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Review

The role of Rho GTPases in disease development

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Abstract

The functionality and efficacy of Rho GTPase signaling is pivotal for a plethora of biological processes. Due to the integral nature of these molecules, the dysregulation of their activities can result in diverse aberrant phenotypes. Dysregulation can, as will be described below, be based on an altered signaling strength on the level of a specific regulator or that of the respective GTPase itself. Alternatively, effector pathways emanating from a specific Rho GTPase may be under- or overactivated. In this review, we address the role of the Rho-type GTPases as a subfamily of the Ras-superfamily of small GTP-binding proteins in the development of various disease phenotypes. The steadily growing list of genetic alterations that specifically impinge on proper Rho GTPase function corresponds to pathological categories such as cancer progression, mental disabilities and a group of quite diverse and unrelated disorders. We will provide an overview of disease-rendering mutations in genes that have been positively correlated with Rho GTPase signaling and will discuss the cellular and molecular mechanisms that may be affected by them.

Keywords: Rho GTPase; Signaling; Disease; Cancer; Neurological disorder

1. Introduction

The family of Rho GTPases comprise a large subfamily of the Ras-superfamily of GTPases. Among all Rho GTPases, Rac1 (Ras-related C3 botulinum toxin substrate 1), Cdc42 (cell division cycle 42) and RhoA (Ras homologous member A) have been studied most extensively. Through the work of many laboratories the role, that Rho GTPases play in cellular processes as diverse as polarization, cell-cell and cell-matrix adhesion, membrane trafficking, cytoskeletal and transcriptional regulation and cell proliferation has made them a group of crucial regulators with a very general relevance (comprehensively reviewed in Hall, 1998; Van Aelst and D'Souza-Schorey, 1997).

As is the case for small GTPases in general, Rho GTPases are guanine nucleotide binding proteins, which cycle between an active GTP-bound and an inactive GDP-bound state, and are subject to distinct control mechanisms. In the inactive state, Rho GTPases are associated with a class of negative regulators, the Rho GDP dissociation inhibitors (GDIs), that stabilize the GDP-bound form of the GTPase and sequester them in the cytoplasm. Their active state is promoted by positive regulators called GDP/GTP exchange factors (GEFs) that (a) tether a given GTPase to a distinct subcellular location and (b) by virtue of their signature tandem Dbl homology (DH)/pleckstrin homology (PH) domain exchange GDP moieties associated with the inactive GTPases for GTP. As a consequence, a conformational switch is induced. This in turn renders the GTPase active and allows it to initiate a productive signaling complex with one of several effector proteins. This instigates an information flow to different cellular destinations via different molecular pathways with different physiological outcomes. The active GTP-bound state is counteracted by negative regulators, the GTPase activating proteins (GAPs), that catalyze the intrinsic ability of a small GTPase to hydrolyze the bound GTP-moiety to GDP (hence the name guanosine tri-phosphatases). Thus, effector binding is reversed and signaling activity halted, causing the biochemical system to come full circle. Understanding this biochemical basis for the function of GTPases has greatly benefited research and lead to the development of constitutively active (GTPase-deficient) and dominant negative (nucleotide

Abbreviations: ALS, amyotrophic lateral sclerosis; CDK, cyclin-dependent kinase; DH, Dbl homology; Dia, diaphanous; ECM, extracellular matrix; ERM, ezrin/radixirilmoesin; FGD, faciogenital dysplasia; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; MRLC, myosin regulatory light chain; MRX, X-chromosome linked mental retardation; PAK, p21 activating kinase; PAR, partitioning defective; PH, pleckstrin homology; ROCK, Rho-associated kinase; WASP, Wiskott–Aldrich syndrome protein

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exchange-defective) mutants that lock a respective GTPase in the GTP- or GDP-bound state. The introduction of such mutants into diverse experimental systems allows for either overactivation or functional deletion of a specific GTPase.

There is a growing list of disease-causing mutations in genes that have been associated with Rho GTPase signaling by means of functional prediction or insights obtained by direct biochemical analysis. These include GEFs, GAPs and effector proteins that appear to be part of quite diverse signaling networks. Surprisingly, though, aberrations in only a single gene encoding a Rho GTPase itself, namely the RhoH gene, have been described thus far to be a putative cause of lymphoma development (see below). Other mutations that may inactivate a Rho gene or lead to an overactive version of the resulting protein due to a lack of extensive screening or functional redundancy have either escaped detection or simply are lethal. This latter possibility is underscored by the fact, that mouse embryos whose Rac1 or Cdc42 genes have been deleted by gene-targeted mutation die early in development (Sugihara et al., 1998; Chen et al., 2000). It may also reflect the multifunctional nature of Rho GTPases. Loss-of-function or constitutive gain-of-function mutations in many Rho GTPases thus may interfere with a number of different cellular processes. Based on our current understanding and dependent on the precise physiological circumstances and cell-types under investigation, a single Rho GTPase can affect a diverse array of phenomena implicated in a cell's specific biology. In addition, there is also continued speculation that Rho-type GTPases need to cycle between their active and inactive states in order to exert their complete physiological potential (discussed by Symons and Settleman, 2000).

On the other hand, it is likely that regulators and effectors of Rho GTPases are expressed and act in a more specific manner, be it in the context of a specific cell-type, tissue-type or developmental process. Genetic loss-of-function mutations in these regulators or effectors, even in form of a germline mutation, may result in a weaker impairment than loss of the respective GTPase itself. The continuing revelation of novel genetic lesions in genes encoding Rho regulators and effectors fully supports this possibility.

The following sections summarize examples of disease processes whose underlying genetic alterations affect the normal function and regulation of Rho GTPases. We examine the importance of such mutations in cancer progression, mental disabilities and other disorders.

2. Rho GTPases in cancer progression

The evidence that directly implicates aberrant Rho-signaling activity in cancer has been obtained either by means of mutations uncovered in various genes encoding Rho-signaling components, or by screening and interference protocols that focus on specific aspects of cancer biology. For a detailed summary of the biological understanding of the pivotal role of Rho-type GTPases in cancer-related processes such as cell-proliferation, migration, invasion and metastasis, we refer the reader to some excellent recent reviews (Symons, 1996; Schmitz et al., 2000; Price and Collard, 2001; Pruitt and Der, 2001). See also Fig. 1.

2.1. Rho GTPases in transformation

In contrast to the 'classical' oncogenic Ras proteins, such as N-Ras, H-Ras and K-Ras, that are frequently mutated in human cancers (Bos, 1988), to date only a single sequence alteration has been detected in a gene encoding a Rho GTPase. In a set of patients diagnosed with non-Hodgkin's lymphoma a t(3;4)(q27;p11–13) translocation was found to be responsible for the pathogenic progression of the disease. Upon closer examination of this locus, a genetic fusion of a gene encoding the newly designated RhoH/TTF GTPase with the LAZ3/BCL3 gene was detected. While a single distinct transcript of the fused loci could be amplified by reverse transcriptase–polymerase chain reaction, it remains elusive whether the promotion of the leukemic aberration is in some way attributable to the RhoH portion of the fusion product (Preudhomme et al., 2000). However, the possibility of RhoH as a player in tumorigenesis has more recently been strengthened by the finding that the RhoH locus is subject to an aberrant hypermutation activity present in B cells. It has been speculated that the somatic hypermutation process that normally generates variable (V) regions in immunoglobulin chains can become misdirected to other genomic loci making it a causative mechanism for about 50% of diffuse large-cell lymphomas. This misdirected hypermutation activity among other loci also has been demonstrated to target the RhoH gene and in particular its upstream non-coding portion. The latter implies the possibility of a change in transcript and/or protein levels contributing to malignant lymphoblastic alterations (Pasqualucci et al., 2001). Again, it has to be mentioned that the specific cellular and biochemical consequences of a mutated RhoH gene as well as the functions of the normal counterpart still have to be determined.

Despite RhoH being the only example of a Rho-specific mutation in humans thus far, it has been clearly demonstrated that Rho-family members play an important role in Ras-induced transformation. Evidence for this has been provided by experiments utilizing constitutively active and dominant negative mutant forms of Rho GTPases in focus forming assays and their ability to enable growth in soft agar as well as tumor formation in nude mice. These protocols allow growth factor-independent proliferation and contact-independent growth. Different laboratories have shown that the activation of distinct Rho GTPases is an essential step towards the fully transformed phenotype triggered by an activated Ras oncogene. Specifically, a dominant negative form of Rac1 markedly inhibits the focus forming activity invoked by Ras in fibroblasts but cannot interfere with an activated, membrane-targeted version of the Raf-kinase

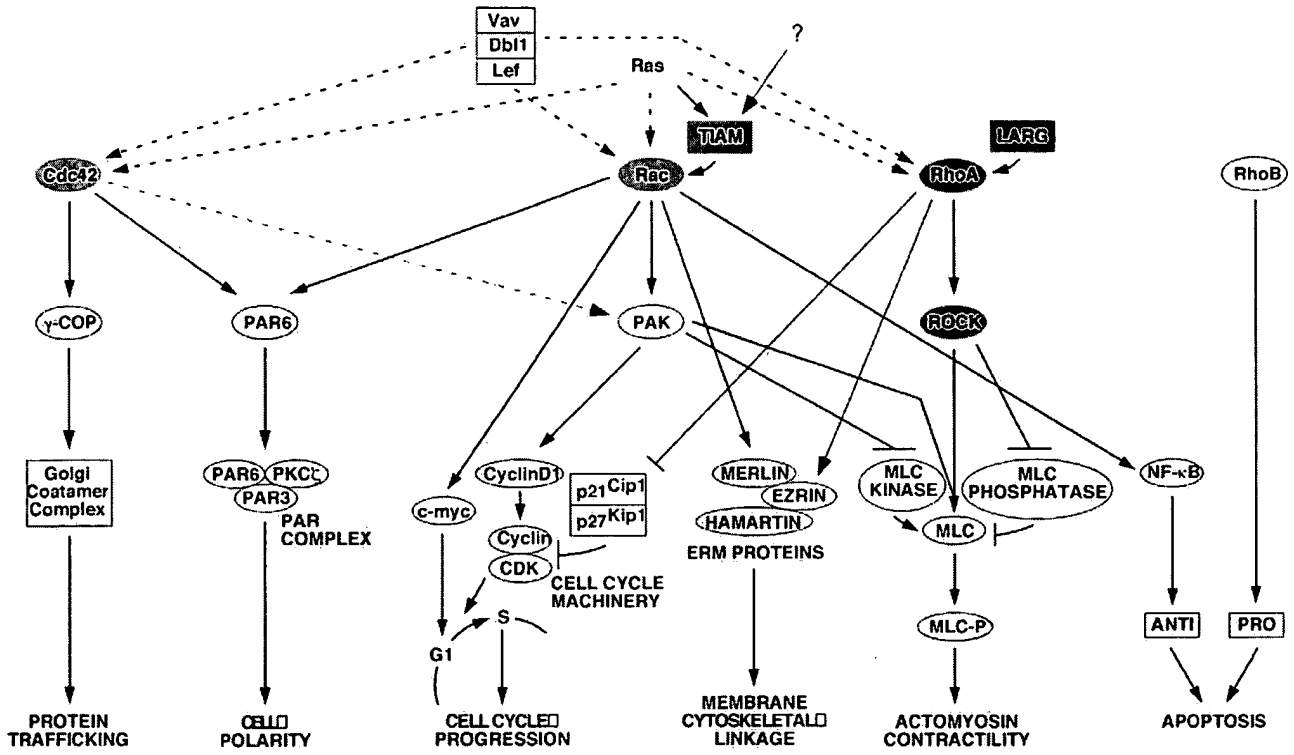

Fig. 1. Potential pathways in which Rho GTPases influence tumorigenic processes. The individual elements depicted in the figure are discussed in Section 2. Boxes, that are highlighted with colors represent small Rho GTPases or molecules that have been found mutated in cancers. Solid lines indicate links, for which unequivocal evidence has been presented.

which acts as the Ras-effector enhancing MAPK activity. In contrast, an activated form of Rac1 significantly enhances the focus forming potential of membrane-targeted Raf (Qiu et al., 1995a). These observations led to the conclusion that Rac is activated by Ras via a Raf-independent pathway. Further studies corroborated these findings and identified RhoA as a second Rho-family GTPase mediating the effects of Ras. Khosravi-Far et al. and Qiu et al. observed that the focus forming activity of a weakly transforming Raf-1 mutant was greatly enhanced when co-introduced together with constitutively active forms of Rac1 or RhoA into fibroblasts. In contrast, the dominant negative forms of Rac1 and RhoA could partially block Ras transformation (Khosravi-Far et al., 1995; Qiu et al., 1995b). In another study, Symons and co-workers added Cdc42 to the list of participants in that they demonstrated that Cdc42 enabled Ras-transformed cells to grow independent of anchorage (Qiu et al., 1997). Additional studies involving Cdc42 suggest that the ability of a GTPase to cycle could be an essential requirement for its transforming potential. In elegant experiments, Lin et al. introduced an F28L mutation into Cdc42, which subjects the GTPase to spontaneous GTP-binding coupled to a wild-type GTP-hydrolysis rate. Cycling in this mutant is therefore greatly accelerated. When stably expressed in fibroblasts, $Cdc42^{F28L}$ yields features of transformed cells including a reduced serum-dependency and anchorage-independency (Lin et al., 1997). Taken together, the genetic interactions summarized above led to the view that Ras not only triggers the activities of the Raf-MAPKK-MAPK cascade but also acts through an alternative route that stimulates Rho-type GTPases which in turn contribute as ancillary factors to the fully transformed phenotype. Furthermore, a picture emerged in which each of the investigated Rho GTPases appears to contribute to a different aspect of the transformed phenotype.

In contrast to the failure to localize mutations in Rho GTPase genes (with the exception of RhoH), there is a growing literature reporting transcriptionally upregulated levels of particular Rho proteins in many types of cancers including those as genetically diverse as those occurring in colon, breast, lung and pancreas (Suwa et al., 1998; Fritz et al., 1999; Jordan et al., 1999; Clark et al., 2000; Schnelzer et al., 2000; van Golen et al., 2000; Kamai et al., 2001). The findings of Jordan and colleagues in particular describe the overexpression of a spliced isoform of Rac1, namely Rac1B, in colorectal tumors, indicating the possibility that oncogenic regulation of Rho GTPases can also occur on the level of mRNA-processing (Jordan et al., 1999; Schnelzer et al., 2000). Recent genomic analysis, combining a protocol selecting for highly metastatic melanoma cells in mice with microarray analysis identified the RhoC gene as a promoter of metastatic behavior. Subsequently, directed expression studies verified this finding (Clark et al., 2000; van Golen et al., 2000). Whether this observation will hold true in

human tissue systems as well, and what the concrete cellular consequences of this phenomenon are, await to be seen. It is noteworthy, however, that, in ductal adenocarcinomas of the pancreas as well as in inflammatory breast cancer cells, RhoC expression was shown to be upregulated (Suwa et al., 1998; van Golen et al., 2000). An intriguing finding is that of the novel Cdc42-like GTPase Wrch-1 as a Wnt-1 transcriptional target (Tao et al., 2001). The Wnt signaling pathway, mostly through mutational analysis of the Wnt-family genes as well as the APC and β-catenin genes, has been implicated in tumorigenesis (Peifer and Polakis, 2000; Polakis, 2000). The contribution of Wrch-1 to the list of Wnt-responsive genes may help understand the mechanisms employed by Wnt-signaling to induce morphological changes, interfere with cell–cell adhesion and cell–extracellular matrix interactions and other phenomena. It also raises the interesting possibility of Rho GTPases functioning as a general class of mediators in multiple oncogenic pathways.

While all these changes in transcript and protein levels are likely to correlate with an elevation in signaling activity in vivo, there are few studies that directly assess the activation state of particular Rho GTPases under transforming conditions. One study that does address this issue is the one by Mira and colleagues who were able to monitor an increase in Rac3 activity in transformed and highly proliferative breast epithelial cells derived from human cancer samples (Mira et al., 2000).

2.2. *Rho GTPase regulation by upstream activators*

As another line of evidence that implicates the Rho-family of GTPases in certain aspects of cancer development, members of the Dbl-family of Rho-GEFs have been classified as oncoproteins. Again, this classification is mainly based on the classical transformation assays mentioned above. The Dbl-family contains such well-investigated members as the founding member of the family, Dbl, the Vav, TIAM, Lcf proteins and others. They all share the tandem Dbl homology and PH homology motifs are now a hallmark feature for Rho-specific activator proteins. Distinct Dbl-family members may show either overlapping activity towards more than one of the Rho GTPases, or specifically activate only one of them (see Fig. 1). Activating deletions and point mutations usually in regulatory regions of the molecules evoke their focus forming abilities. For example, in the case of Dbl and Vav, the N-termini of the proto-oncoproteins have been found to repress their catalytic activities. Accordingly, deletion of these N-terminal sequences can relieve this repression and result in a constitutive activation of the GEF function. The structural basis for some of these autoinhibitory mechanisms and their release has been convincingly disclosed in an NMR spectroscopic analysis focusing on the Vav paradigm of Tyr-phosphorylation-mediated activation (Aghazadeh et al., 2000). Stimulation of cells with particular extracellular agents ensues rapid and transient phosphorylation of Vav which in turn abolishes its autoinhibitory function with a concomitant increase in GEF-activity (for review, see Bustelo, 2000).

Upon closer inspection of the available literature, we noticed that naturally occurring gain-of-function mutations in patient-derived material have so far only been described for Dbl and the more recently identified LARG and TIAM genes. The Dbl oncogene was originally isolated from a human diffuse B-cell lymphoma DNA in a coupled gene transfer/transformation assay (Srivastava et al., 1986), while LARG (leukemia-associated Rho guanine exchange factor) was isolated as a fusion partner of the MLL (mixed lineage leukemia) gene in acute myeloid leukemia (Kourlas et al., 2000). Subsequently, work by two groups produced biochemical evidence for LARG's identity as a Rho activator (Fukuhara et al., 2000; Reuther et al., 2001). The protein's tandem DH/PH domain appears to be specific for RhoA since LARG can activate the Rho-dependent serum response factor (SRF) but not Rac/Cdc42-dependent Jun kinase (JNK) signaling (Reuther et al., 2001). Taya et al., in a recent study, revealed that the PDZ domain of LARG associates with the carboxyterminal tail of the insulin-like growth factor-1 (IGF-1) receptor (Taya et al., 2001). Elaborating on this finding, the authors further produce evidence that IGF-1 stimulation of epithelial MDCKII cells results in an activation of RhoA and its effector kinase ROCK to give rise to enhanced stress fiber formation. Since the IGF-1 receptor/LARG complex forms constitutively also in the absence of IGF-1 ligand but on the other hand RhoA activity is triggered by exposure to IGF-1, the authors speculate that LARG activation is subject to an as yet unidentified IGF-1-dependent mechanism (Taya et al., 2001). It is also possible, that the RGS (regulator of G-protein signaling) domain harbored by the protein serves to couple G-protein-coupled receptors (GPCRs) and heterotrimeric G proteins of the G alpha(12) family to Rho-dependent signaling (Fukuhara et al., 2000). These initial biochemical studies, together with the finding that LARG is expressed in all human tissues examined, will surely promote further interest in this molecule.

TIAM (T-cell invasion and metastasis gene) was found in an extensive screen, that was designed to isolate invasion-promoting genes in T cells (Habets et al., 1994). Subsequently, TIAM was demonstrated to display Rac-specific exchange activity (Michiels et al., 1995). In light of earlier experiments that showed Rac to promote the spread of cancer cells and metastasis in nude mice (Qiu et al., 1995a), these data proposed a TIAM/Rac-dependent, invasion-promoting signaling pathway. This opened a different perspective on the role of Rho-proteins in cancer progression, namely their function in metastasis and invasion in aggressive cancer cells. This property of some Rho GTPases has since been reiterated in different experimental settings (e.g. the one described for the finding of RhoC above). In fact, analysis of the TIAM locus in a set of renal-cell carcinomas revealed several mutant TIAM alleles. One of these mutations, A441G, maps to the protein's N-terminal pleckstrin homology domain and could potentially interfere with

the proper membrane localization and functional activity of the protein. In focus forming assays, the A441G mutation can convert TIAM into a transforming molecule which further underlines its relevance in malignant processes (Engers et al., 2000). Interestingly, a putative metastasis suppressor, nm23H1, which was isolated from murine melanomas by subtraction cloning associates with TIAM and seems to negatively regulate cell motility of tumor cells of murine and human origin (Leone et al., 1991; Kantor et al., 1993; MacDonald et al., 1996). This activity correlates with a downregulation of Rac1 (Otsuki et al., 2001). Thus nm32H1 may prove to be an interesting physiological link to TIAM and Rac regulation during invasion and metastasis. It has to be mentioned that depending on the specific cell type under investigation (Hordijk et al., 1997) and the particular ECM composition faced by those cells (Sander et al., 1998), TIAM can also generate an adhesion-promoting effect. In epithelial MDCK cells, its expression may even reverse the loss of E-Cadherin mediated adhesion induced by oncogenic Ras (Hordijk et al., 1997). Therefore, the involvement and regulation of TIAM in invading and metastasizing cancer cells might be more complex than the current data suggest.

2.3. Downstream effectors and effector pathways

What is the nature of the underlying signaling pathways mediating distinct aspects of cellular transformation triggered by Rho GTPases and which are the immediate downstream effectors regulating them?

A necessary requirement for a cell to transit from a normal to a transformed state is the dysregulation of its cell cycle machinery. From earlier observations, it became evident that cyclin D1, as a key factor required for the G1/S transition whose levels oscillate throughout the cell cycle, is transcriptionally upregulated by the Ras-induced Raf/MEK/ERK pathway (Cheng et al., 1998; Kerkhoff and Rapp, 1998). Subsequently, it has been demonstrated that Rac can also stimulate activity at the cyclin D1 promoter (Westwick et al., 1997; Gille and Downward, 1999). Cyclin D1, as a functional consequence of its enhanced synthesis, teams up with its cognate cyclin-dependent kinase partners CDK4 and CDK6. The major substrate for the phosphorylation-activity of the complex is the Retinoblastoma protein whose subsequent degradation triggers progression through the G1-phase of the cell cycle (Sherr and Roberts, 1999). It is worth noting, that a survey of human breast cancer etiology in patients has revealed the cyclin D1 gene to be amplified in 20% of the cases examined (Dickson et al., 1995). Moreover, cyclin D1 protein levels appeared to be elevated in more than 50% of mammary carcinomas (Bartkova et al., 1994; Gillett et al., 1994; McIntosh et al., 1995). Further corroborating the relevance of this transcriptional target, Yu et al. (2001) observed that cyclin D1-deficient mice are able to resist cancer development induced by the Ras and Neu oncogenes. It is conceivable that Rac could either contribute to the full oncogenic upregulation of cyclin D1 transcription by Ras or that it leads to a sustained promoter activity. However, Rac can also activate the cyclin D1 gene in Ras-independent pathways. Consistent with this, activation of integrin signaling has recently been demonstrated to modulate the levels of cyclin D1 protein in a Rac-dependent manner in order to trigger proliferation of primary cells in culture. Surprisingly, in this case Rac appears to influence cyclin D1 expression on the translational rather than the transcriptional level (Mettouchi et al., 2001). In a recent publication Welch et al. presented a more refined concept highlighting differential effects of Rho GTPases on cyclin D1 expression (Welsh et al., 2001). Whereas, in their studies Rac1 and Cdc42-emanating mechanisms induced cyclin D1 early in G1-phase of the cell cycle, Rho-activity may ensure sustained cyclin D1 expression throughout G1 in an ERK-dependent fashion. Apart from cyclin D1 as a transcriptional target, the expression of the cell-cycle regulator c-myc, induced by PDGF, has been demonstrated to rely on Rac activity and to occur independently of Ras. Together, this information suggests, that a single Rho GTPase might affect the cell cycle via a number of different pathways (Chiariello et al., 2001).

Rho, on the other hand, seems to impinge on another class of cell cycle regulatory proteins, namely the cyclin/CDK complex inhibitors $p21^{Cip1}$ and $p27^{Kip1}$. Evidence for this comes from the observations, that (1) Rho activity interferes with $p21^{Cip1}$ in that it suppresses its induction (Olson et al., 1998), and (2) $p27^{Kip1}$ degradation is facilitated by Rho (Weber et al., 1997; Hu et al., 1999). Hence, as in the case of cyclin D1 upregulation, these Rho-specific phenomena lead to a stabilization of cyclin/CDK activity, which consequently is thought to accelerate progression through the cell cycle.

To date, it remains unresolved, which of the many effector molecules of the Rho-family GTPases unambiguously relay an oncogenic potential in vivo and which of the activated signaling pathways are the ones responsible. There have been no activity-modifying mutations described in any of the known Rho GTPase effector proteins in cancerous cells. One of the candidates relaying the Rac signal to the cell cycle machinery in order to achieve transformation, however, is the Ste20-like p21 PAK-kinase. PAK kinases were first identified by Manser et al. who performed biochemical overlay assays with the intention to isolate novel Rac1 and Cdc42 effector proteins (Manser et al., 1994). A catalytically inactive form of PAK can effectively suppress Ras-induced transformation in some cell lines (Tang et al., 1997) and, it has been shown that PAK activity correlates with cyclin D1 promoter induction (Joyce et al., 1999). Similarly, the function of Rac3 in the proliferation of breast tumor cells appears to correlate with increased PAK activity (Mira et al., 2000). PAK, apart from its correlation to cell proliferative events, also has been related to aspects of microfilament reorganization and invasiveness in breast cancer cells. In this setting, a kinase-dead version of PAK1

can suppress the motile and invasive phenotypes of otherwise highly invasive human MDA-MB435 cells (Adam et al., 2000).

Although these data suggest a role for PAK in tumorigenesis/metastasis, it remains unclear as to whether PAK is dependent on Rac-activity to exert its effect. It has been demonstrated that PAK-independent pathways emanating from Rac are likely to participate in promotion of Rac's transforming potential. It also has been shown that Rac-mutants engineered to no longer bind PAK, but that still tether other effectors, can persist to evoke transformation of fibroblasts (Joneson et al., 1996; Lamarche et al., 1996; Westwick et al., 1997). This is suggestive of the notion, that Rac can target the cell cycle apparatus through PAK-dependent or PAK-independent pathways or by multiple pathways operating simultaneously in a given cell.

The ROCK effector-kinases have been proposed to convey Rho's transforming ability. ROCK-type kinases serve as regulators of the actomyosin network in that they promote phosphorylation of the regulatory light chain of myosin in a Rho-dependent manner. This in turn stimulates contractility of actin filaments, which is the basis of cell motility. A constitutively active version of the kinase can synergize with activated Raf in conventional transformation assays (Sahai et al., 1999). An additional and intriguing piece of evidence for ROCK's role in tumorigenesis has been provided by Narumiya and colleagues who developed and successfully applied a ROCK-specific inhibitor (Y-27632). Highly metastatic Rat MM1 hepatoma cells are able to transmigrate through a mesothelial cell monolayer in a serum and specifically lysophosphatidic acid-dependent manner. This migratory behavior is enhanced by transfection with a dominant positive form of ROCK, while conversely, it is inhibited by a ROCK mutant acting as a dominant negative version of the kinase. Treatment with Y-27632 can block Rho-mediated invasion in MM1 cell cultures and, moreover, can interfere with the dissemination of MM1 cells when implanted into the peritoneal cavity of syngeneic rats (Itoh et al., 1999). Y-27632 also interferes with the transforming activities of RhoA itself and the ones of the Rho-activators Dbl and Net (Sahai et al., 1999). In the first experiments with human cells, Y-27632 was able to partially abolish the anchorage-independent growth of two colorectal carcinoma cell lines and in another assay inhibited human prostate cancer cells from disseminating when introduced into immune-compromised mice (Somlyo et al., 2000). Whether this inhibitor will prove to be genuinely ROCK-specific and whether the data from these animal experiments can be extrapolated to the invasion of human metastatic cells in human tissues has to be shown by further careful investigation.

Recently, it was found that ROCK-dependent phosphorylation of the Ezrin/Radixin/Moesin (ERM)-family member Ezrin is a necessary requirement in the transformation process induced by oncogenic Dbl-proteins (Tran Quang et al., 2000). ERM-proteins are thought to link the actin cytoskeleton to membrane regions undergoing dynamic morphological changes. Net or Dbl oncogene expression can interfere with the normal cell-cell contact inhibitory effects on proliferation, which may contribute to their oncogenic potentials. Introduction of an Ezrin mutant that does not serve as a ROCK-substrate anymore may re-establish a contact-inhibitory mechanism under those circumstances. This clearly is indicative of the possibility, that Rho/ROCK signaling constitutes the link between Dbl- and Net-activity as a malignant stimulus and an ERM-protein function at the membrane as one of its outputs. The observation, that ROCK-dependent Ezrin phosphorylation can be blocked by Y-27632 addition nicely supplemented this connection (Tran Quang et al., 2000). Ezrin also has been found to interact with Hamartin, the gene product of the TSC1 tumor suppressor gene locus (Lamb et al., 2000) as well as RhoGDI and the Dbl oncoprotein (Takahashi et al., 1997, 1998). Together, these insights strongly emphasize the importance of Rho/ROCK mediated signaling in cancer progression.

Another correlation between Rho GTPase signaling and an ERM-like protein is the one that has been established more recently between Rac and Merlin by Shaw and co-workers (Shaw et al., 2001). Merlin is the product of the NF2 gene, mutations in which predispose humans and mice to the development of Neurofibromatosis type II (NF2). Since mutations in the NF2 gene that abolish Merlin synthesis are facilitating or causing tumor formation, NF2 has been defined as a tumor suppressor gene (Gutmann et al., 1997; Gusella et al., 1999). In an endeavor to shed light on the molecular network NF2/Merlin is embedded in, Shaw et al. have produced evidence that the functional state of Merlin is controlled by Rac. The expression of constitutively active Rac or its activators Dbl and Tiam promotes the phosphorylation of Merlin at the critical C-terminal Ser-518 residue. This modification is thought to inhibit the 'closed' conformation of the molecule that relies on the head-to-tail interaction between N- and C-terminal residues within the Merlin protein and rather promotes its 'open' conformation. Since it is the closed state that exhibits a negative growth regulatory function in vitro and in vivo (Sherman et al., 1997), the release of its inhibitory functions by activated Rac may provide a contribution to Rac's tumorigenic potential. This possibility has been strengthened by additional experimentation that showed Rac-induced transformation to be drastically reduced by concomitant expression of Merlin. NF2-deficient fibroblasts also display features that strongly resemble those elicited by Rac, such as membrane ruffling and an increase in the number of intracellular vesicles (Shaw et al., 2001). These data taken together suggest an interesting model. Merlin might be linking events at the plasma membrane with the cell cycle machinery and in particular cell cycle-promoting cyclin D1 levels. In a setting, where no functional Merlin protein is present like in NF2 mutant cells, this block is genetically abolished. Alternatively, in cases where a Rac-dependent mechanism interferes with the growth-inhibitory properties of wild-type Merlin, its function is biochemically disrupted. Although speculative, this scenario could offer a

partial explanation for the Rac-provoked cell cycle stimulation.

A potentially oncogenic mechanism involving a Rho GTPase and impacting on vesicle trafficking has recently brought to light (Wu et al., 2000). Whereas Rho GTPases have been implicated in various steps of membrane trafficking (for review see Ridley, 2001), the significance for this in oncogenic processes remains ill-understood. Cdc42 has been implicated by Wu et al. in the functional modulation of the Golgi coatamer complex. The coatamer complex serves to shuttle cargo from the endoplasmic reticulum to the Golgi apparatus and Cdc42 directly associates with the γ-COP subunit (Wu et al., 2000). The same authors found that Cdc42 has to target γ-COP in order to generate the transformed phenotype characteristic of the 'fast cycling' $Cdc42^{F28L}$ mutant mentioned above. This observation provides the first clue for the involvement of membrane trafficking in cellular transformation (Wu et al., 2000). It was known earlier, that Cdc42 localizes to the Golgi compartment and that it affects several transport steps such as the exit of apical and basolateral proteins from the trans-Golgi network and the endocytic transport to the basolateral plasma membrane in polarized cells (Erickson et al., 1996; Kroschewski et al., 1999; Musch et al., 2001). While the precise consequences of the Cdc42/γ-COP interaction are not well understood, it may be a first molecular link for Cdc42's association with the Golgi compartment and its local effects therein.

Another door that just has been opened concerns the role of Rho GTPases in the establishment of cellular polarity. While the general relevance of Rho GTPases in these processes has been demonstrated, the discovery of the PAR6 protein as a Rac1 and Cdc42 effector contributes a first molecular insight. PAR6 is a constituent of the Par-6/Par-3/PKCζ complex that is vital for the establishment of cellular polarity in diverse systems (Joberty, 2000 #167; Johansson, 2000 #170; Lin, 2000 #168; Qiu, 2000 #138). In mammalian cells, PAR3, PAR6 and PKCζ are associated with tight junctional structures and Rac1/Cdc42 engagement of the PAR3/PAR6 complex is thought to participate in tight junction formation in part likely by inducing PKCζ activity (Joberty et al., 2000; Qiu et al., 2000). Overexpression of a kinase-dead PKCζ version in MDCK cells causes mislocalization of PAR3 correlating with a severe impairment of tight junctional biogenesis (Suzuki et al., 2001). Moreover, ectopically expressed PAR6 enhances the transforming potential of Rac1 (Qiu, 2000 #138). These experiments clearly indicate that the PAR3/PAR6/PKCζ complex is sensitive to changes in the ratio of its components and to ectopically altered levels of its kinase activity. Since tight junctional integrity is required for the maintenance of cell polarity and proper polarity is abolished in transformed cells, targeting of the Par-6/Par-3/PKCζ complex by activated Rac1 or Cdc42 could contribute to malignant transformation. The activation of atypical PKC isoforms including PKCζ previously has been correlated to the control of cell growth and survival. Ectopic amounts of PKCζ can counteract apoptotic signals and experimental down-regulation of PKCζ levels and activity impair cell proliferation and activation of NF-κB transcription which in many situations prevents apoptosis (Berra et al., 1993; Dominguez et al., 1993; Diaz-Meco et al., 1996).

It has been demonstrated that the cell survival machinery appears to be directly affected by Rho GTPases. Like oncogenic Ras (Finco et al., 1997; Mayo et al., 1997), Rac, Cdc42 and Rho positively regulate the transcription at NF-κB-dependent promoters (Sulciner et al., 1996; Perona et al., 1997) and thus may prevent cells driven to a transformed state from undergoing apoptosis. NF-κB also appears to be required for the transforming abilities of the RhoA and Cdc42 activators Dbl and Dbs (Whitehead et al., 1999). These pathways can also interfere with Ras-induced apoptosis (Joneson and Bar-Sagi, 1999) and the expression of dominant active Rac can prevent suspension-induced apoptosis ('anoikis') of epithelial cells (Coniglio et al., 2001) as well as apoptosis triggered by serum deprivation of fibroblastic cells (Ruggieri et al., 2001). It will be interesting to see, whether PKCζ activation in polarity determining complexes by Rac1 and/or Cdc42 will connect through a distinct signaling pathway to antiapoptotic and proliferative events like e.g. activation of NF-κB. Of note is the observation that Rac2-deficient cells derived from gene targeted mice display significantly reduced survival in the presence of growth factors as compared to control cells. This property, furthermore, has been correlated with a failure to induce the survival factors Akt and BAD/Bcl-XL (Yang et al., 2000).

Targeted deletion and transgenic expression experiments in mice revealed diverse roles also for Rho GTPase genes and their products in mediating apoptosis and cell survival in a context-dependent manner (Cleverley et al., 2000; Costello et al., 2000; Liu et al., 2001a). Mutant mouse embryo fibroblasts (MEFs) that have been derived from RhoB-deficient mice when transformed with the H-Ras and adenovirus E1A oncogenes displayed a significantly elevated resistance to apoptosis after being exposed to DNA-damaging reagents as compared to non-targeted cells. It appears that RhoB has taken on a unique role among the Rho GTPases. In contrast to the developmental necessity of the Rac1 and Cdc42 genes and their products, the RhoB gene is dispensable for development and normal physiological aspects of mouse biology (Sugihara et al., 1998; Chen et al., 2000; Liu et al., 2001b). However, the proapoptotic function that has been ascribed to RhoB may be required under conditions that generate cellular stress as exemplified by the exposure to DNA- and microtubule damaging compounds or under cell transforming circumstances (Liu et al., 2001a,b). In fact, the relatively high turnover rates the RhoB protein is subjected to often are counteracted by transcription of the RhoB gene (Jahner and Hunter, 1991; Fritz et al., 1995). The currently available data hence strongly argue for a proapoptotic function for RhoB that eliminates damaged cells with a potential to elude normal cell cycle control and thus to become a cancer-

ous hazard for the organism. This property clearly distinguishes RhoB physiology from the anti-apoptotic aspects of Rac1.

In contrast, in hemopoietic cells Rho has clearly been correlated with cell survival signaling (Costello et al., 2000). The thymocyte-specific lck promoter was used to drive the expression of the Rho-inhibitor C3 (bacterial toxin C3 transferase from Clostridium botulinum). C3 selectively ADP-ribosylates and inactivates RhoA, B and C (Boquet, 1999). Under these conditions, pre-T cells that have not undergone β selection to assemble vβ-chains into functional pre-T cell receptors (TCRs) are subject to massive apoptosis. This cell death phenomenon is abrogated in a p53 loss of function situation suggesting a p53-dependent mechanism that is governed by Rho-activity. After formation of functional pre-T cell receptors, however, Rho appears to promote survival via a p53-independent and BCL-2-sensitive pathway (Costello et al., 2000). Another study by Cleverley et al. shows that the same mice, when 4 to 8 months old, develop aggressive thymic lymphoblastic lymphomas (Cleverley et al., 2000). Analysis of the tumors revealed a lack of heterogeneity in the vβ-chain of the T-cell receptor (TCR) complex which led the authors to hypothesize a monoclonal origin of the malignant cells. Since lck-driven C3 expression alone does not ensue tumor formation per se, it is likely that an additional genetic alteration underlies the observed clonal expansion. Given that all 3 Rho isoforms are interfered with by C3 and taken in particular the RhoB functions described above into consideration, it is conceivable, that concomitant inactivation of RhoB could favor tumor development by inhibiting apoptosis in malignant clones.

In summary, the significance of Rho GTPase signaling impinges on various aspects of oncogenesis (see Fig. 1). As exemplified in the aforementioned, cell cycle progression in tumor cells, their adhesive properties, migratory and invasive behavior and escape from apoptotic extinction all seem to be affected by Rho GTPase activity. Whereas a scaffold emerges in which a particular Rho GTPase is linked to specific physiological effects, defined signaling pathways still need to be worked out. As is the case with Y-37632 as a ROCK inhibitor, other participants in specific signaling pathways may serve as drug targets to inhibit malignant processes that depend on Rho GTPase activity. In addition, given, that dysregulations provoked by Rho GTPases phenocopy cancerous aspects of oncogenesis in general, it is tempting to speculate that Rho signaling might be contributing to other oncogenic pathways as well. The identification of Wrch-1 as a transcriptional target of oncogenic Wnt signaling is a first example for such interdependencies.

3. An emerging r le for Rho GTPases in neur degenerative disorders

Rho GTPases are currently gaining increasing attention for their involvement in different classes of neurodegenerative disorders that reflect vital functions of Rho GTPases in diverse aspects of the nervous system. Over the past few years, Rho GTPases have been implicated in neuronal processes including neuronal migration and polarization, axon guidance and dendrite formation, as well as synaptic organization and plasticity (comprehensively reviewed in (Luo, 2000). Given the large number of GEFs and GAPs shown to be functional and/or expressed in the nervous system, and others that are predictable on the basis of the available genome sequences, it is likely that Rho GTPase activity is intrinsic to various signaling pathways involved in the regulation of neuronal processes. Many of these pathways will be responsive to extracellular cues and stimuli to evoke cytoskeletal rearrangements, which underlie detectable morphological adjustments in cells of the nervous system (Fig. 2).

One complex of quite heterogeneous neuropathological disorders constitutes the nonsyndromic, X-chromosome linked forms of mental retardation (commonly referred to as MRX or XLMR). MRX affects approximately 1 in 500 males and represents about 25% of all genetically manifested cases of mental retardation. The only feature in individuals affected by MRX is an impairment of their cognitive functions. While no gross anatomical alterations in brain structures have been observed in MRX-inflicted individuals, closer histological inspection has revealed that the hippocampus and certain cerebellar ventricles often are increased in size. In contrast, the cerebral cortex often appears reduced in size when compared to unaffected control tissue (Reiss et al., 1991; Reiss et al., 1994). Detailed microscopic analysis showed that the dendritic spines in the affected regions are thinner and more elongated in MRX-patients. Furthermore, the synaptic contacts they establish are more reminiscent of those made by immature spines (Rudelli et al., 1985; Hinton et al., 1991). Spine synapses are considered to relay the majority of functional excitatory synaptic communication. Moreover, they are regarded as the structures displaying most of the ongoing synaptic plasticity that determines the efficacy of 'learning and memory' processes (Matus, 1999). Moreover, spine morphology is particularly dependent on actin structures and processes that continuously remodel them. These are processes that in many respects require the directed regulation and activity of the Rho-family of GTPases (Luo, 2000).

Through means of positional cloning, about ten genes associated with MRX have been identified thus far, offering the first mutational basis to study the specific genetics and biochemistry involved in MRX. Among these, three MRX genes encoding oligophrenin-1, αPIX/Cool-2/ARHGEF6 and PAK3 represent elements of potential Rho GTPase-dependent signaling pathways that are active in neurons. Others, such as IL1RAPL and TM4SF2 being additional identified MRX-genes and FMR1 being associated with Fragile X mental retardation syndrome (FraX) may be physiologically linked to Rho-function.

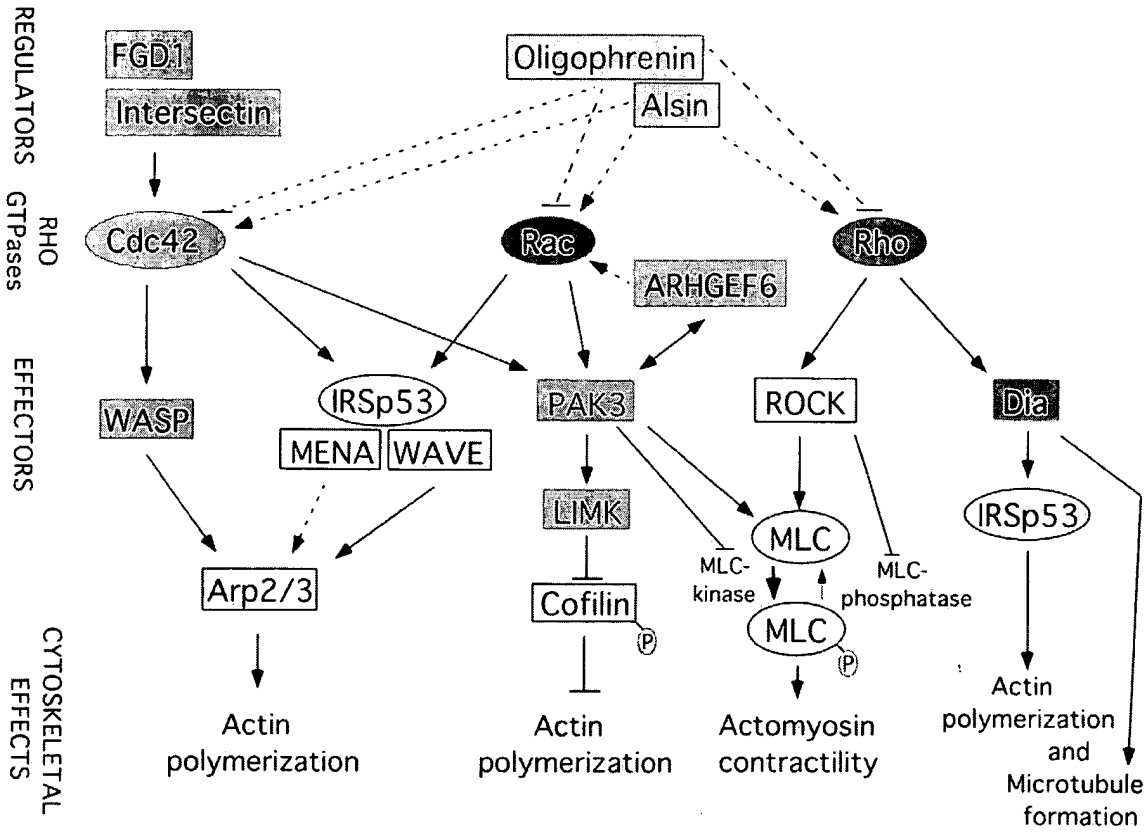

Fig. 2. The putative signaling networks in which Rho GTPase related genes involved in mental retardation and other disorders may affect the cytoskeleton. The specific genes and their products are referred to in Sections 3 and 4.

Besides the genetic lesions underlying various forms of mental retardation, the recent description of mutations in a gene encoding a potential RhoGEF, called alsin, as a cause for amyotrophic lateral sclerosis (ALS) presents another example for the involvement of Rho GTPases in neuronal processes. ALS is a fatal neurological disorder in which motor neurons progressively degenerate. The finding that a putative RhoGEF is mutationally inactivated in this disease suggests that impairment of proper Rho GTPase signaling may be an important step in the etiology of neurodegenerative phenomena.

Finally, another candidate molecule that is being discussed as a disease-related Rho GTPase signaling component is intersectin. The intersectin gene is located on human chromosome 21 and the biochemical properties revealed so far indicate that its protein product could contribute to the neuronal defects observed in Down syndrome.

3.1. Mental retardation-associated genes

3.1.1. Oligophrenin-1

The first Rho-related MRX gene to be identified was the oligophrenin-1 gene. It encodes a putative RhoGAP protein with a canonical RhoGAP catalytic domain, which is likely to have a negative effect on Rho GTPase signaling. Two different mutations in the oligophrenin-1 gene have been isolated, both of which are supposed to lead to a loss-of-function phenotype (Antonarakis and Van Aelst, 1998; Billuart et al., 1998). So far, it remains incompletely understood as to which of the Rho GTPases is the physiologically relevant target of oligophrenin-1 in vivo although the protein exerts GAP activity towards RhoA, Cdc42 and Rac1 in vitro. This observation emphasizes a role for oligophrenin-1 as a negative regulator of Rho GTPase activity. Nevertheless, more specifically designed experiments will have to address the in vivo substrate preference, subcellular localization and the nature of the protein network it is part of to understand the disease-causing role of the isolated mutations. It is conceivable that oligophrenin-1 also fulfills extra-neuronal functions, since it is expressed in tissues other than the brain, albeit at lower levels (Billuart et al., 1998). However, loss of oligophrenin-1 function in humans does not give rise to any discernible phenotypes in addition to cognitive impairments in affected individuals suggesting that a loss of oligophrenin-1 in other tissues may be compensated for by functional redundancy.

3.1.2. PAK3

A second Rho-related MRX-gene is PAK3. Two different mutations in the PAK3 gene have been isolated in two

MRX-pedigrees. As described earlier, PAK kinases have been defined as Rac1- and Cdc42-specific effector molecules that activate pathways downstream of activated Rho GTPases (Manser et al., 1994, see Fig. 2). PAK3 is highly expressed in MRX-relevant brain regions, namely the cortex and hippocampus, suggesting that PAK3 kinase function is required either for the development of these regions, or for the inherent synaptic plasticity of synaptic spines in specialized regions with particular cognitive tasks (Allen et al., 1998). PAK kinases, according to a current model, are thought to exist in a cytoplasmically dormant state. Upon recruitment by an upstream factor, which could be activated Rac1 or Cdc42, the autoinhibitory interaction between the N-terminal regulatory domain and the C-terminal catalytic domain is disrupted. This switches PAK into an active conformation capable of auto- and substrate phosphorylation (Aghazadeh et al., 1998; Bagrodia and Cerione, 1999; Buchwald et al., 2001). A point mutation present in a multiplex pedigree abrogates the Ser/Thr-directed kinase function by inserting a premature stop-codon (Allen et al., 1998). In another MRX pedigree, the missense mutation R67C has been implicated as a cause for moderate to severe mental retardation. This amino acid substitution resides in the predicted Rho GTPase-interaction motif of PAK3 and hints to the relevance of the GTPase/PAK interaction (Bienvenu et al., 2000). Of note, mutations in the *Drosophila* PAK homolog have been implicated in developmental aspects of the nervous system such as neurogenesis and axonal pathfinding (Melzig et al., 1998; Hing et al., 1999; Newsome et al., 2000). Imperfectly understood downstream signaling pathways relay PAK-activity to the actin cytoskeleton and possibly the nucleus. The identification of the PAK-relevant kinase targets in MRX will greatly contribute to our understanding of the physiological correlation between Rho GTPase activities and cognitive functions.

So what are the molecular components linking PAK to cytoskeletal alterations in neurons? Different proteins have been hypothesized to serve as substrates for PAK's kinase activity in general and particular neuronal situations. PAK, as part of its activation mechanism, phosphorylates itself (Manser et al., 1994). Furthermore, LIMK (or Lim-domain-containing protein kinase) has been identified by Edwards et al. as a potential downstream mediator of PAK (Edwards et al., 1999). Upon phosphorylation by PAK, LIM-kinase in turn phosphorylates, and thus inactivates, the actin depolymerizing factor Cofilin (Arber et al., 1998; Yang et al., 1998). This study suggests that PAK can connect Rac/Cdc42 activity to LIM-kinase in the regulation of actin cytoskeletal dynamics. Interestingly, hemizygosity of the LIM-kinase 1 encoding locus on chromosome 7q11 is correlated with the autosomal William's syndrome, a disorder comprised of a complex of phenotypes which affects about 1 in 20,000 births. Features of this condition include weakened visuospatial constructive cognition and varying severities of mental retardation (Frangiskakis et al., 1996). Another substrate of PAK is MLCK (myosin light chain kinase), which exerts kinase activity towards the myosin regulatory light chain (MRLC) (Sanders et al., 1999). MRLC-phosphorylation by MLCK is thought to stimulate actin/myosin motor complexes. Since MLCK is inactivated by PAK, this event is believed to counteract actin/myosin motor activity. In addition, MRLC has also been proposed to be a direct PAK target (Sells et al., 1999). Most of these initial biochemical observations have come from the study of fibroblasts or epithelial cell lines. Thus, these different findings will have to be re-evaluated as to their importance in neurons in order to elucidate the relevant mechanisms involving the loss of PAK3 in MRX.

3.1.3. ARHGEF6

Another intriguing addition to the list of MRX-causing mutations is the ARHGEF6 gene discovered by Kutsche and colleagues (Kutsche et al., 2000). The ARHGEF6 gene product is identical to the previously discovered αPIX/Cool-2 protein isolated as a PAK-binding partner by means of biochemical co-purification and yeast-2-hybrid approaches. In fact, a small protein family containing αPIX/Cool-2 itself, βPIX/p85Cool-1 and its smaller splice variant p50Cool-1, has emerged (Bagrodia et al., 1998, 1999; Manser et al., 1998). Interestingly, αPIX/Cool-2 also contains the typical tandem DH/PH motif that marks it as a potential RhoGEF (Fig. 2). The potential for an interaction between αPIX/Cool-2 and PAK is mediated by an SH3 domain in the former and an unconventional SH3-binding site in the latter protein. αPIX/Cool-2, when co-expressed with PAK, can trigger PAK's kinase activity (Bagrodia et al., 1998; Daniels et al., 1999). Attempts to monitor directly the levels of [^{3}H]GDP released from Rac1 or Cdc42 did not unequivocally reveal an exchange activity towards either of the GTPases. However, in in vitro assays, αPIX/Cool-2 can increase the amount of GTP-bound Cdc42, whereas its sibling βPIX/p85Cool-1 can increase GTP levels bound to Rac1. This activity, however, is significantly lower than that of the well-studied RhoGEF Dbl (Bagrodia et al., 1998, 1999; Manser et al., 1998; Daniels et al., 1999). Taken together, the expression and biochemical experiments performed so far suggest that αPIX/Cool-2 can enhance PAK recruitment and activity but do not give an unambiguous answer as to the exact molecular mechanisms governing the GTPase/αPIX/Cool-2/PAK complex. Most probably, other factors and precise subcellular conditions dictate the function of this complex in various in vivo settings. As in the case of PAK3, comprehension of the concrete cellular effects of the mutations found in ARHGEF6 requires further study.

3.1.4. IL1RAPL, TM4SF2 and FMRP

Other that the genes described above that are direct regulators or effectors of Rho GTPases, there is a group of mental retardation genes that are not evident Rho-signaling components but may still be indirectly linked to the function of Rho GTPases. IL1RAPL, TM4SF2 and FMRP code for a

novel member of the interleukin-1 (IL-1) receptor family, a protein of the tetraspanin family of membrane proteins and an RNA-binding protein, respectively (Carrie et al., 1999; Zemni et al., 2000). It has been reported that IL-1, an inflammatory cytokine, can stimulate Cdc42 through its cognate IL-1 RI and RII receptors in fibroblasts. As a consequence, Rac and Rho GTPases also become activated. In fact, Cdc42 activity is absolutely required for IL-1-induced actin polymerization and remodeling processes (Puls et al., 1999). Exposure of HeLa cells to IL-1 results in the activation of RhoA, which in turn leads to the formation of stress fibers. Moreover, IL-1 RI, in a biochemical affinity purification protocol, was found to be complexed with RhoA and Rac1 (Singh et al., 1999). It will be interesting to see, whether the newly identified MRX-related IL1RAP protein also will involve Rho GTPases as part of its general signaling potential in the brain.

Tetraspanins have been observed in protein complexes with adhesion molecules such as α- and β-integrins. The TM4SF proteins CD151, CD81 and CD63 have been found to be associated with α3β1-integrins in neurites and growth cones of human NT2N cells. When treated with antibodies against CD151 and CD81, neurite outgrowth in NT2N cells grown on α3β1 integrin-specific extracellular matrix molecules (ECM) was greatly impaired. Under these conditions, neurite number, length and extension rate where all affected (Stipp and Hemler, 2000). As there appears to be a general requirement for Rho GTPase activity in axon and neurite outgrowth and retraction, as well as a functional connection between integrin and Rho signaling it would not be surprising to find that TM4SF/integrin complexes function in concert with the Rho GTPases (Jalink et al., 1994; Luo et al., 1994). Intriguingly, in a recent study, a TM4SF-family member was netted as a binding partner of oligodendrocyte-specific protein (OSP/Claudin-11), a member of the expanding Claudin family, which is responsible for establishing tight junction-equivalent structures in myelin-forming oligodendrocytes of the CNS. For this reason, the respective TM4SF protein was named OAS for OSP/Claudin-11 associated protein 1 (Tiwari-Woodruff et al., 2001). Furthermore, β1-integrin subunits were recovered as another constituent of OAS/OSP complexes (Tiwari-Woodruff et al., 2001). Loss of OSP/Claudin-11 in gene-targeted mice results in the absence of tight junction-like structures in CNS myelin sheaths, which induces neurological abnormalities (Gow et al., 1999). Interference experiments using OAP-1- and OSP/Claudin-11-specific antibodies in primary oligodendrocytes effectively impaired migration, whereas, overexpression of OAP-1 or OSP/Claudin-11 caused an oligodendrocytic cell line to overproliferate in culture (Tiwari-Woodruff et al., 2001). In light of the well-established role of Rho proteins in determining the morphology and function of tight junctions in epithelial cells (Jou et al., 1998), these data may hint at the possibility of oligodendrocytic defects being important in the genesis of MRX. It remains to be seen which specific cell type TM4SF2 will be functional in. Its wide spread expression pattern so far does not exclude any of the mentioned possibilities, all of which may well be molecularly and physiologically linked to Rho GTPase signaling.

Finally, a potentially intriguing role for Rac1 signaling in the development of the fragile X mental retardation syndrome has been suggested (Schenck et al., 2001). FMRP (fragile X-linked mental retardation protein) is the product of the FMR1 gene, mutations in which manifest themselves in the Fragile X mental retardation syndrome (FraX). FraX can be distinguished from non-syndromic X-linked mental retardation in that it is also associated with other phenotypes such as macroorchidism, large ears, prominent jaws and a high-pitched jocular speech in affected individuals (Hagerman, 1996; Imbert et al., 1998). In FraX, the FMR1 gene is transcriptionally silenced due to a hypermethylated CGG repeat expansion in the sequence encoding the 5′-untranslated region (Imbert et al., 1998). FMRP harbors nuclear export and import signals, as well as multiple mRNA binding motifs (Ashley et al., 1993; Siomi et al., 1993; Eberhart et al., 1996; Sittler et al., 1996; Tamanini et al., 1999). Thus it may serve to shuttle specific mRNAs between the nucleus and the cytoplasm. The finding that the concentration of FMRP is high in neurons and particularly within dendritic processes suggests that it may direct its cargo mRNAs to a specialized cellular compartment for more efficient translation (Devys et al., 1993; Weiler et al., 1997; Jin and Warren, 2000). More recently, FMRP has been shown to associate with CYFIP1 (cytoplasmic FMRP interacting protein 1), a component of the synaptosome (Schenck et al., 2001). In fact, CYFIP1 was a known protein that earlier had been described as a Rac1 interacting protein, termed p140Sra-1, which was observed to be associated with Rac-induced cortical actin filaments and to cosediment with F-actin (Kobayashi et al., 1998). It is tempting to speculate that activated Rac1, together with CYFIP1, tethers FMRP to developing spines to spatially control the translation of spine-relevant cargo mRNAs. Rac1 activation, in this scenario, may result from activation of receptors for neurotransmitters in spine synapses. Mutational down-regulation of FMRP may interfere with such translational processes, which ultimately produces FraX-associated phenotypes.

As illustrated above, a number of MRX genes are either evident components of Rho GTPase signaling cascades or may be indirectly linked to the activities of Rho GTPases. Since each of the cloned MRX genes accounts for only 0.5–1.0% of the total MRX cases (Chelly, 2000), one can predict a considerable number of additional MRX genes to surface in the future. It is very likely that among these MRX genes additional components of Rho GTPase signaling pathways will be identified. Tying in MRX-defects on their physiological level with the molecular details of Rho GTPase signaling will be a major challenge. Experiments addressing whether the Rho GTPase-related MRX genes and their products found thus far may cooperate in a common path-

way is an interesting starting point. Also the development of animal models carrying targeted disruptions in MRX genes and transgenes that can be conditionally expressed will be beneficial to these aims. Defining these Rho GTPase-controlled pathways and integrating them into a general neuronal signaling network will contribute to understanding the molecular details of cognitive processes.

3.2. Motor neuron degeneration and Down syndrome

3.2.1. Alsin/ALS2 in amyotrophic lateral sclerosis (ALS)

A major breakthrough in the search of genetic mutations causing familial amyotrophic lateral sclerosis (ALS) has been the recent cloning of a locus on chromosome 2q33 encoding a putative novel RhoGEF by two independent groups (Hadano et al., 2001; Yang et al., 2001; Fig. 2). Accordingly, one of the groups coined the term alsin (ALSin) for the respective protein product.

ALS is a progressive paralytic disorder that affects motor neurons in different regions of the nervous system. Due to genetic lesions, upper motor neurons (UMN) in the motor cortex as well as lower motor neurons (LMN) in the brainstem and spinal cord are rendered dysfunctional and degenerate. This causes the affected individuals to die most frequently from respiratory failure, often within 3 years after onset of the disease. Whereas 90% of the ALS cases are sporadic, 10% are of familial origin. The familial cases harbor genetically transmitted, usually autosomal dominant mutations. In a small percentage of familial ALS (ALS2) the first clinical manifestations are detectable before the age of 25, with the disease developing at a slower pace than in the rest of the familial ALS patients.

The first, and for a long time only, mutated ALS-gene identified was the one coding for Cn/Zn superoxide dismutase (SOD1) (Rosen, 1993). SOD1's catalytic activity reduces the amount of free radicals in virtually all cell types and ALS-specific mutations are thought to render SOD1 hyperactive rather than inactive, suggesting the necessity for a delicate balance in the number of free radicals for motor neuronal processes. According to estimations based on multiple mapping efforts, the SOD1 locus only accounts for about 20% of familial ALS cases arising from autosomal dominant mutations. Aberrations in other genes were eagerly awaited.

The alsin gene was the second locus to be involved in the neurodegenerative processes underlying ALS, in particular the juvenile form of ALS, ALS2. The discovery of alsin as a putative Rho GTPase activator could open new vistas on the ill-defined biochemical pathways that if impaired can cause ALS. The microdeletions found in the alsin gene disrupt its coding unit, and thus generate loss-of-function alleles (Hadano et al., 2001; Yang et al., 2001). The alsin protein, apart from a typical tandem DH/PH domain, contains two types of motifs that are also present in other GTP/GDP exchangers for Ras GTPases. Aligned in its N-terminus are three copies of the RCC1-like domain (RLD), which was originally described for the Ran GTPase-specific exchange factor RCC1. RCC1 associates with chromatin and regulates the nuclear-cytoplasmic shuttling of Ran. At its C-terminus, alsin harbors a VPS9 domain that is shared by exchange factors mediating vacuolar protein sorting in yeast in the case of VPS9 itself, or endocytic trafficking in the case of VSP9 domain-containing eukaryotic GEFs. In addition, alsin contains an array of MORN (membrane occupation and recognition nexus) motifs that very recently have been implicated in the membrane association of junctophilins (Takeshima et al., 2000). Junctophilins comprise a novel protein family that is present at junctional complexes established between the plasma membrane and extensions of the endoplasmic/sarcoplasmic reticulum, which are features of excitable cell types and are thought to mediate communication between the plasma membrane and intracellular ion channels.

All of the listed consensus motifs may contribute to alsin's specific subcellular location and function, but the specific role of alsin in motor neurons is illusive at this point. Pivotal issues concerning the Rho GTPase-specificity and the sub-neuronal localization of alsin still need to be resolved to solidify the basis for further speculation. However, in light of the disease phenotype that is produced by alsin-specific mutations, namely the degeneration of motor neuronal circuits, it is intriguing to speculate on a more general role of Rho GTPase-dependent signaling for the maintenance of neuronal pathways. The disruption of such pathways might provoke neurodegenerative processes.

3.2.2. Intersectin as a potential component in Down syndrome

The intersectin-encoding gene on chromosome 21q22 has been speculated to contribute to the malignant aspects of Down syndrome (Guipponi et al., 1998). Intersectin, which represents yet another member of the RhoGEF family has been implicated into endocytic events and mitogenic processes and might even link the two (Fig. 2). Mechanistically, intersectin stimulates Cdc42 and thus regulates N-WASP/Arp2/3-dependent actin polymerization, possibly at sites of active endocytosis (Hussain et al., 2001; O'Bryan et al., 2001). The classification of intersectin as a genuine disease molecule may be premature at this point, but the localization of the encoding gene on chromosome 21 and elevated expression levels of intersectin-specific mRNA in patients suffering from Down syndrome make it an interesting molecule in the context of trisomy 21 research (Guipponi et al., 1998; Pucharcos et al., 2000, 2001).

4. Other potentially Rho GTPase-related disabilities

4.1. FGD1 (faciogenital dysplasia)

By conventional means of forward genetics, the FGD gene has been cloned and revealed to be the mutated

locus causing faciogenital dysplasia, also known as Aarskog–Scott syndrome (Pasteris et al., 1994). The discovery of additional mutant FGD-alleles since then has confirmed the role of the gene in the development of the disease (Orrico et al., 2000; Schwartz et al., 2000). Faciogenital dysplasia is an X-linked developmental disorder and individuals are of disproportionately short stature and suffer from facial, skeletal and urogenital abnormalities. The FGD gene product, FGD1, was predicted to function as a Rho-specific GEF (Fig. 2), and the disease-causing mutation at first identified by Pasteris et al. was predicted to insert a premature stop codon into the region encoding the protein's DH-function. FGD1, apart from the DH-motif also contains the juxtaposed PH-domain typical of Rho GEFs, as well as a number of SH3-binding regions and an additional C-terminal PH-domain (Pasteris et al., 1994). Subsequently, Zheng and colleagues have demonstrated that FGD1 exerts its activity specifically on Cdc42 (Zheng et al., 1996). Further dissection of the FGD1 molecule determined that the DH-domain alone can induce G1 progression when introduced into fibroblasts, but that both the DH and the contingent PH sequence are needed to induce a Cdc42-specific response of the actin cytoskeleton, namely the formation of filopodia and microspikes (Nagata et al., 1998). This result suggests that proper recruitment of FGD1 to actin-relevant subcellular sites depends on proper PH-function. Interestingly, mutational analysis of another FGD pedigree by Orrico et al. (2000) has revealed a R610Q amino acid substitution. Arg-610 resides in the PH-domain of the DH/PH-motif and appears to be a conserved residue in PH-domains that are involved in inositol-phosphate (InsP)-binding (Ferguson et al., 1995; Salim et al., 1996). Together, these results suggest that FGD1 is targeted by specific InsP-species to distinct membranes where it exerts its GEF activity towards Cdc42. To date, no upstream activating mechanism for FGD1-specific signaling has been described but the strong similarity between the PH-domain harboring Arg-610 and the one of the β-adrenergic receptor kinase (ARK) suggests that PtdIns-4,5-P_2 and/or PtdIns-3,4,5-P_3 might mediate the recruitment of FGD1 to membrane domains. ARK has been shown to associate with these InsP species (Rameh et al., 1997).

4.2. WASP (Wiskott–Aldrich syndrome)

Two other genetic disorders in which mutations affect Cdc42 signaling are the X-linked Wiskott–Aldrich syndrome (WAS) and the related allelic X-linked thrombocytopenia (XLT). As with FGD and the MRX genes, the gene causing WAS and XLT is located on the X-chromosome, rendering recessive alleles (approximately 50 different mutations have been detected so far in the WAS gene) to phenotypic expression (Derry et al., 1995; Kolluri et al., 1995; Kwan et al., 1995; Villa et al., 1995; Wengler et al., 1995; Zhu et al., 1995). Defects are restricted to hematopoietic lineages and include microthrombocytopenia and recurrent infections because of dysregulated T- and B-cell functions. The observations that neutrophils are enabled in their proper chemotactic response and that B cells do not show their typical response to polysaccharides, together with the severe cytoskeletal defects observed in T cells and platelets, suggested an actin-related role for the WAS gene product. Derry et al. succeeded in cloning the gene responsible for WAS and through rigorous screening assembled the first set of WAS-specific mutations including nonsense, missense and frame shift mutations (Derry et al., 1994). Subsequently, in biochemical overlay assays designed to find novel Cdc42-effectors, Symons et al. isolated WASP (for WAS protein) from human neutrophils (Symons et al., 1996). WASP appeared to bind exclusively to GTP-loaded Cdc42 and not to Rac1 or RhoA, and was traced in high concentrations to polymeric actin structures. Through the work of several groups a picture emerged, in which Cdc42, in concert with WASP, stimulates the Arp2/3 complex which in turn enables actin filaments to nucleate from germination centers (for review see Welch, 1999; Fig. 2). This activity is required for morphological cell shape changes and cell migration, processes that are impaired in WASP-deficient hematopoietic cells. Accordingly, WASP transcripts were found in compartments of the immune system, such as the spleen, thymus and lymphocytes (Derry et al., 1994). Notably, a homologue of WASP, namely N-WASP is expressed ubiquitously, with particularly high expression levels in the nervous system, and fulfills WASP functions in cells other than those of hematopoietic origin (Miki et al., 1996). In subsequent experiments, N-WASP was shown to be an indispensable element in the Cdc42-dependent pathway leading to the formation of filopodia in fibroblasts (Miki et al., 1998). WASP, moreover, appears to be subject to precise regulatory mechanisms to guarantee its proper function. This has become even more apparent when Devriendt et al. recently reported that another hematopoietic deficiency, namely X-linked severe congenital neutropenia (XLN), is caused by a WAS mutation that generates a constitutively activated WASP protein (Devriendt et al., 2001). A single amino acid substitution at position 270 from Leu to Pro in the autoinhibitory domain of WASP was detected, and based on structural data by Kim and co-workers (Kim et al., 2000) is shown to map to the Rho GTPase binding domain (Kim et al., 2000; Devriendt et al., 2001). These findings underscore the in vivo importance of the Cdc42/WASP interaction once more.

4.3. Diaphanous (non-syndromic deafness)

Another example of a gene encoding a disease-related Rho GTPase effector is Diaphanous 1 (also known as DFNA1). An effort to identify the autosomal dominant mutation responsible for nonsyndromic deafness led to the cloning of a gene on chromosome 5q31 that turned out to be homologous to the *Drosophila diaphanous* gene (Lynch et al., 1997). The first clue hinting at a possible function for

diaphanous came from another source. Watanabe and co-workers, in a quest for RhoA-specific binding partners, identified p140mDia/mDia1, the mouse orthologue of the diaphanous protein (Fig. 2). They further showed that mDia1 was not only tethered to activated Rho by its N-terminus, but that it also serves as a ligand for Profilin via its formin homology (FH1) domain (Watanabe et al., 1997). This interaction is believed to stimulate actin remodeling at particular locations in the cell in response to Rho activation. Indeed, in a follow-up study, mDia1 turned out to work in concert with ROCK to induce stress fibers in transfected fibroblasts (Watanabe et al., 1999). Interestingly, mDia1 caused the alignment of actin together with microtubule filaments in a coordinated bipolar fashion when transfected into HeLa cells. This activity was ascribed to the C-terminal FH2 domain of the molecule and suggests mDia1/diaphanous as an actin/microtubule coordinating factor downstream of Rho (Ishizaki et al., 2001). Similarly, Palazzo et al. found mDia1 to colocalize with microtubules and to trigger the formation and orientation of stable microtubules in serum-starved fibroblasts (Palazzo et al., 2001). Deafness, arising from a mutation in the diaphanous gene is associated with a sensorineural cochleosaccular dysplasia of the membranous structures of the inner ear. It has been speculated that diaphanous may act in a pathway linking integrins to the actin cytoskeleton. Mutations in $\alpha 8\beta 1$ integrins, diaphanous and myosinVIIa (as well as other myosins) all cause deafness, indicating that the wild-type proteins could organize and/or maintain cytoskeletal structures in hair cells of the inner ear (Lynch et al., 1997; Richardson et al., 1999; Littlewood Evans and Muller, 2000; for a review about deafness-related genes see Muller and Littlewood-Evans, 2001).

4.4. A potential role for Rho GTPases in Tangier disease

In the last 'case study' presented in this review, Rho GTPase function has been correlated with Tangier disease. The basis of this disease is an impaired cholesterol efflux (CE) mechanism, resulting in abnormally low high density lipoprotein (HDL) plasma levels and an accumulation of cellular cholesteryl esters. Thus, CE is an essential process that purges excess cholesterol from cells and provides an important protection against arteriosclerosis. Examination of fibroblasts and macrophages from Tangier patients by cDNA subtraction techniques revealed a substantial down-regulation of Cdc42 levels in affected cells. Accordingly, Hirano et al. found that introduction of a dominant negative form of Cdc42 into epithelial MDCK cells could decrease CE efficiency and that dominant active Cdc42 could increase it (Hirano et al., 2000). In addition, RhoA, RhoB, RhoG and Rac1 levels appeared to be elevated in fibroblasts derived from Tangier patients (Utech et al., 2001). Whether Rho GTPases play a direct role in Tangier disease, and how a particular Rho GTPase affects aspects of CE, remains elusive, but future research into this subject may well shed light onto yet another aspect of Rho GTPase signaling.

4.5. Rho GTPases and bacterial infections

Through work over the last few years, it has become clear that the manipulation of Rho GTPases is a step in a number of disease-causing bacterial infections. Rho GTPases are manipulated by bacterial toxins from quite different origins and of different make and function. For more detail, we refer to recent reviews that treat this subject with much greater depth (Lerm et al., 2000; Stebbins and Galan, 2001). To summarize the recent research, Rho GTPases are targeted by basically three classes of toxins that display fundamentally different activities. The first class contains the Rho GTPase-inactivating C3 exotoxins and LCC (large clostridial toxin), toxins that act as ADP-ribosyltransferases and glucosyltransferases, respectively. The covalent attachment of ADP-ribosyl-moieties by the C3-transferases of *Clostridium botulinum*, *Staphylococcus aureus* and other infectious bacteria blocks the ability of RhoA, B and C to interact with Rho-specific GEFs and thus prevents activation of these GTPases. Glucosylation of Rho at Thr-37 and Rac and Cdc42 at Thr-35 by the large LCC toxins (larger than 250 kDa) which is produced by *Clostridium* spp., interferes with nucleotide binding and coordination of the nucleotide-linked Mg^{2+}-ion. This in turn inhibits the association of effector molecules with the same region of the GTPases and abrogates the stimulation of downstream signaling pathways. The second class of toxins contains the Rho GTPase-deamidating CNF (cytotoxic necrotizing factor) agents from *Escherichia coli* and DNT (dermonecrotic toxin) toxins from *Boretella* spp. CNF toxins deamidate Gln-63 in Rho and Gln-61 in Rac and Cdc42. Since these residues are essential for GTP-hydrolysis and therefore inactivation of the Rho GTPases, this renders them constitutively active. This results in an overactivation of particular Rho-dependent signaling pathways. DNT toxins are transglutaminases that constitutively activate Rho by adding an amine to Gln-63.

A particularly interesting class of toxins is the one of 'injected' toxins, that due to a specialized 'typeIII' secretion system are directly introduced from *Yersinia* and *Salmonella* bacteria into their host cells. 'Injected' toxins mimic cellular GEFs and GAPs to a degree that allows the invading bacterium to first activate Rho GTPases, such as Rac1 and Cdc42 in the case of SopE from Salmonella spp. This ensues remodeling of the actin cytoskeleton to promote bacterial entry. Post internalization, another class of 'injected' toxins, exemplified by SptP from *Salmonella* spp. and YopE from *Yersinia* spp. is produced, this time to reversibly inactivate Rho GTPases by mimicking host GAPs. Thus, deregulation of cytoskeletal structures is reversed and the host cell saved for the future benefit of the invaders.

Recent structural analysis has unraveled the basis for some of these mechanisms. It appears that through conver-

gent evolution *Salmonella* spp. and *Yersinia* spp. have developed proteins that, although they do not share the tertiary structures of the enzymes they mimic, nevertheless display contact surfaces and identical residues at critical positions that allow for catalysis (Lerm et al., 2000; Stebbins and Galan, 2001). The corruption of Rho GTPase activities in the interest of pathogenic infection can be regarded as a redundant theme and the different strategies that have been evolutionarily employed by bacteria to this end are diverse and fascinating. The observation that overactivation as well as underactivation of Rho GTPases occurs in the course of bacterial attacks suggests that Rho GTPase signaling is being exploited in some cases and disrupted in others. In this sense, the strategies used by *Salmonella* spp. seem even more sophisticated. The C3-transferase from *C. botulinum* already has been used successfully as an experimental tool to inhibit Rho-function in diverse systems (see e.g. Cleverley et al., 2000). It is conceivable, that other Rho GTPase-specific toxins will be introduced as more generally applicable reagents into research that pertains to clinically relevant questions.

5. Conclusions and future perspectives

Given the complexity of Rho GTPase signaling and the multiple cellular and developmental aspects involving and requiring the function of Rho GTPases, there is a very strong possibility that many more disease-causing mutations in genes encoding Rho-related signaling molecules will be uncovered in the future. The available annotated genome sequences suggest a vast number of genes for Rho GTPase-specific regulators, but many of them remain biologically unexplored to date. Emerging areas of interest in which the contribution of Rho GTPases is currently being investigated include mechanisms of viral infection and various organ- and tissue-specific diseases, such as ventricular myocyte hypertrophy underlying some dysfunctions of the heart. However, due to lack of compelling evidence that would definitively prove the corruption of a Rho GTPase-related signaling molecule, we chose not to elaborate on these processes.

The following points illustrate some of the possibilities that will influence future research to provide us with a better concept of how Rho GTPases function in numerous disease aspects. (1) The findings that Wnt- and Ras-signaling may feed into Rho-signaling to manifest some Rho GTPase-dependent phenotypes emphasize the interdependency and collaboration of different signaling pathways during the progression of a given disease. Other disease-relevant pathways will be linked to Rho-signaling in the years to come. Adhesion-triggered signaling in various forms is a good candidate for this. (2) Rho-specific activities in the establishment of cellular polarity and cell-cell adhesive properties, as well as their impact on vesicle trafficking, may be of importance for the development of different diseases. Also for this reason, studies in these areas merit further attention. (3) Along the same lines, the MRX genes encoding components and potential components of Rho GTPase signaling pathways that have been identified thus far will have to be functionally connected to an operational network. The establishment of mouse model systems will help in understanding the contribution of these genes and their interactions on a physiological level.

It is evident that the biological activities of the disease genes elaborated upon in this review will exceed the scope of the context in which they were initially identified. Research addressing these aspects may ultimately also help design strategies for therapeutic intervention and diagnostic purposes. Certainly, the interplay and collaboration between disease research on the one hand, and biochemical, as well as cell biological, research on the other will further help advance these subjects and activate our individual levels of excitement.

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References

Adam, L., Vadlamudi, R., Mandal, M., Chernoff, J., Kumar, R., 2000. Regulation of microfilament reorganization and invasiveness of breast cancer cells by kinase dead p21-activated kinase-1. J. Biol. Chem. 275, 12041–12050.

Aghazadeh, B., Zhu, K., Kubiseski, T.J., Liu, G.A., Pawson, T., Zheng, Y., Rosen, M.K., 1998. Structure and mutagenesis of the Dbl homology domain. Nat. Struct. Biol. 5, 1098–1107.

Aghazadeh, B., Lowry, W.E., Huang, X.Y., Rosen, M.K., 2000. Structural basis for relief of autoinhibition of the Dbl homology domain of proto-oncogene Vav by tyrosine phosphorylation. Cell 102, 625–633.

Allen, K.M., Gleeson, J.G., Bagrodia, S., Partington, M.W., MacMillan, J.C., Cerione, R.A., Mulley, J.C., Walsh, C.A., 1998. PAK3 mutation in nonsyndromic X-linked mental retardation. Nat. Genet. 20, 25–30.

Antonarakis, S.E., Van Aelst, L., 1998. Mind the GAP. Rho, Rab and GDI. Nat. Genet. 19, 106–108.

Arber, S., Barbayannis, F.A., Hanser, H., Schneider, C., Stanyon, C.A., Bernard, O., Caroni, P., 1998. Regulation of actin dynamics through phosphorylation of cofilin by LIM- kinase. Nature 393, 805–809.

Ashley Jr, C.T., Wilkinson, K.D., Reines, D., Warren, S.T., 1993. FMR1 protein: conserved RNP family domains and selective RNA binding. Science 262, 563–566.

Bagrodia, S., Cerione, R.A., 1999. Pak to the future. Trends Cell Biol. 9, 350–355.

Bagrodia, S., Taylor, S.J., Jordon, K.A., Van Aelst, L., Cerione, R.A., 1998. A novel regulator of p21-activated kinases. J. Biol. Chem. 273, 23633–23636.

Bagrodia, S., Bailey, D., Lenard, Z., Hart, M., Guan, J.L., Premont, R.T.,

Taylor, S.J., Cerione, R.A., 1999. A tyrosine-phosphorylated protein that binds to an important regulatory region on the cool family of p21-activated kinase-binding proteins. J. Biol. Chem. 274, 22393–22400.

Bartkova, J., Lukas, J., Muller, H., Lutzhoft, D., Strauss, M., Bartek, J., 1994. Cyclin D1 protein expression and function in human breast cancer. Int. J. Cancer 57, 353–361.

Berra, E., Diaz-Meco, M.T., Dominguez, I., Municio, M.M., Sanz, L., Lozano, J., Chapkin, R.S., Moscat, J., 1993. Protein kinase C zeta isoform is critical for mitogenic signal transduction. Cell 74, 555–563.

Bienvenu, T., des Portes, V., McDonell, N., Carrie, A., Zemni, R., Couvert, P., Ropers, H.H., Moraine, C., van Bokhoven, H., Fryns, J.P., Allen, K., Walsh, C.A., Boue, J., Kahn, A., Chelly, J., Beldjord, C., 2000. Missense mutation in PAK3, R67C, causes X-linked nonspecific mental retardation. Am. J. Med. Genet. 93, 294–298.

Billuart, P., Bienvenu, T., Ronce, N., des Portes, V., Vinet, M.C., Zemni, R., Roest Crollius, H., Carrie, A., Fauchereau, F., Cherry, M., Briault, S., Hamel, B., Fryns, J.P., Beldjord, C., Kahn, A., Moraine, C., Chelly, J., 1998. Oligophrenin-1 encodes a rhoGAP protein involved in X-linked mental retardation. Nature 392, 923–926.

Boquet, P., 1999. Bacterial toxins inhibiting or activating small GTP-binding proteins. Ann. N. Y. Acad. Sci. 886, 83–90.

Bos, J.L., 1988. The ras gene family and human carcinogenesis. Mutat. Res. 195, 255–271.

Buchwald, G., Hostinova, E., Rudolph, M.G., Kraemer, A., Sickmann, A., Meyer, H.E., Scheffzek, K., Wittinghofer, A., 2001. Conformational switch and role of phosphorylation in PAK activation. Mol. Cell. Biol. 21, 5179–5189.

Bustelo, X.R., 2000. Regulatory and signaling properties of the Vav family. Mol. Cell. Biol. 20, 1461–1477.

Carrie, A., Jun, L., Bienvenu, T., Vinet, M.C., McDonell, N., Couvert, P., Zemni, R., Cardona, A., Van Buggenhout, G., Frints, S., Hamel, B., Moraine, C., Ropers, H.H., Strom, T., Howell, G.R., Whittaker, A., Ross, M.T., Kahn, A., Fryns, J.P., Beldjord, C., Marynen, P., Chelly, J., 1999. A new member of the IL-1 receptor family highly expressed in hippocampus and involved in X-linked mental retardation. Nat. Genet. 23, 25–31.

Chelly, J., 2000. MRX review. Am. J. Med. Genet. 94, 364–366.

Chen, F., Ma, L., Parrini, M.C., Mao, X., Lopez, M., Wu, C., Marks, P.W., Davidson, L., Kwiatkowski, D.J., Kirchhausen, T., Orkin, S.H., Rosen, F.S., Mayer, B.J., Kirschner, M.W., Alt, F.W., 2000. Cdc42 is required for PIP(2)-induced actin polymerization and early development but not for cell viability. Curr. Biol. 10, 758–765.

Cheng, M., Sexl, V., Sherr, C.J., Roussel, M.F., 1998. Assembly of cyclin D-dependent kinase and titration of p27Kip1 regulated by mitogen-activated protein kinase kinase (MEK1). Proc. Natl. Acad. Sci. USA 95, 1091–1096.

Chiariello, M., Marinissen, M.J., Gutkind, J.S., 2001. Regulation of c-myc expression by PDGF through Rho GTPases. Nat. Cell Biol. 3, 580–586.

Clark, E.A., Golub, T.R., Lander, E.S., Hynes, R.O., 2000. Genomic analysis of metastasis reveals an essential role for RhoC. Nature 406, 532–535.

Cleverley, S.C., Costello, P.S., Henning, S.W., Cantrell, D.A., 2000. Loss of Rho function in the thymus is accompanied by the development of thymic lymphoma. Oncogene 19, 13–20.

Coniglio, S.J., Jou, T.S., Symons, M., 2001. Rac1 protects epithelial cells against anoikis. J. Biol. Chem. 276, 28113–28120.

Costello, P.S., Cleverley, S.C., Galandrini, R., Henning, S.W., Cantrell, D.A., 2000. The GTPase rho controls a p53-dependent survival checkpoint during thymopoiesis. J. Exp. Med. 192, 77–85.

Daniels, R.H., Zenke, F.T., Bokoch, G.M., 1999. alphaPix stimulates p21-activated kinase activity through exchange factor-dependent and -independent mechanisms. J. Biol. Chem. 274, 6047–6050.

Derry, J.M., Ochs, H.D., Francke, U., 1994. Isolation of a novel gene mutated in Wiskott–Aldrich syndrome. Cell 78, 635–644.

Derry, J.M., Kerns, J.A., Weinberg, K.I., Ochs, H.D., Volpini, V., Estivill, X., Walker, A.P., Francke, U., 1995. WASP gene mutations in Wiskott–Aldrich syndrome and X-linked thrombocytopenia. Hum. Mol. Genet. 4, 1127–1135.

Devriendt, K., Kim, A.S., Mathijs, G., Frints, S.G., Schwartz, M., Van Den Oord, J.J., Verhoef, G.E., Boogaerts, M.A., Fryns, J.P., You, D., Rosen, M.K., Vandenberghe, P., 2001. Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. Nat. Genet. 27, 313–317.

Devys, D., Lutz, Y., Rouyer, N., Bellocq, J.P., Mandel, J.L., 1993. The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. Nat. Genet. 4, 335–340.

Diaz-Meco, M.T., Municio, M.M., Frutos, S., Sanchez, P., Lozano, J., Sanz, L., Moscat, J., 1996. The product of par-4, a gene induced during apoptosis, interacts selectively with the atypical isoforms of protein kinase C. Cell 86, 777–786.

Dickson, C., Fantl, V., Gillett, C., Brookes, S., Bartek, J., Smith, R., Fisher, C., Barnes, D., Peters, G., 1995. Amplification of chromosome band 11q13 and a role for cyclin D1 in human breast cancer. Cancer Lett. 90, 43–50.

Dominguez, I., Sanz, L., Arenzana-Seisdedos, F., Diaz-Meco, M.T., Virelizier, J.L., Moscat, J., 1993. Inhibition of protein kinase C zeta subspecies blocks the activation of an NF-kappa B-like activity in *Xenopus laevis* oocytes. Mol. Cell. Biol. 13, 1290–1295.

Eberhart, D.E., Malter, H.E., Feng, Y., Warren, S.T., 1996. The fragile X mental retardation protein is a ribonucleoprotein containing both nuclear localization and nuclear export signals. Hum. Mol. Genet. 5, 1083–1091.

Edwards, D.C., Sanders, L.C., Bokoch, G.M., Gill, G.N., 1999. Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. Nat. Cell Biol. 1, 253–259.

Engers, R., Zwaka, T.P., Gohr, L., Weber, A., Gerharz, C.D., Gabbert, H.E., 2000. Tiam1 mutations in human renal-cell carcinomas. Int. J. Cancer 88, 369–376.

Erickson, J.W., Zhang, C., Kahn, R.A., Evans, T., Cerione, R.A., 1996. Mammalian Cdc42 is a brefeldin A-sensitive component of the Golgi apparatus. J. Biol. Chem. 271, 26850–26854.

Ferguson, K.M., Lemmon, M.A., Schlessinger, J., Sigler, P.B., 1995. Structure of the high affinity complex of inositol trisphosphate with a phospholipase C pleckstrin homology domain. Cell 83, 1037–1046.

Finco, T.S., Westwick, J.K., Norris, J.L., Beg, A.A., Der, C.J., Baldwin Jr, A.S., 1997. Oncogenic Ha-Ras-induced signaling activates NF-kappaB transcriptional activity, which is required for cellular transformation. J. Biol. Chem. 272, 24113–24116.

Frangiskakis, J.M., Ewart, A.K., Morris, C.A., Mervis, C.B., Bertrand, J., Robinson, B.F., Klein, B.P., Ensing, G.J., Everett, L.A., Green, E.D., Proschel, C., Gutowski, N.J., Noble, M., Atkinson, D.L., Odelberg, S.J., Keating, M.T., 1996. LIM-kinase1 hemizygosity implicated in impaired visuospatial constructive cognition. Cell 86, 59–69.

Fritz, G., Kaina, B., Aktories, K., 1995. The ras-related small GTP-binding protein RhoB is immediate-early inducible by DNA damaging treatments. J. Biol. Chem. 270, 25172–25177.

Fritz, G., Just, I., Kaina, B., 1999. Rho GTPases are over-expressed in human tumors. Int. J. Cancer 81, 682–687.

Fukuhara, S., Chikumi, H., Gutkind, J.S., 2000. Leukemia-associated Rho guanine nucleotide exchange factor (LARG) links heterotrimeric G proteins of the G(12) family to Rho. FEBS Lett. 485, 183–188.

Gille, H., Downward, J., 1999. Multiple ras effector pathways contribute to G(1) cell cycle progression. J. Biol. Chem. 274, 22033–22040.

Gillett, C., Fantl, V., Smith, R., Fisher, C., Bartek, J., Dickson, C., Barnes, D., Peters, G., 1994. Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. Cancer Res. 54, 1812–1817.

Gow, A., Southwood, C.M., Li, J.S., Pariali, M., Riordan, G.P., Brodie, S.E., Danias, J., Bronstein, J.M., Kachar, B., Lazzarini, R.A., 1999. CNS myelin and sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. Cell 99, 649–659.

Guipponi, M., Scott, H.S., Hattori, M., Ishii, K., Sakaki, Y., Antonarakis, S.E., 1998. Genomic structure, sequence, and refined mapping of the

human intersectin gene (ITSN), which encompasses 250 kb on chromosome 21q22.1 → q22.2. Cytogenet. Cell Genet. 83, 218–220.

Gusella, J.F., Ramesh, V., MacCollin, M., Jacoby, L.B., 1999. Merlin: the neurofibromatosis 2 tumor suppressor. Biochim. Biophys. Acta 1423, M29–M36.

Gutmann, D.H., Aylsworth, A., Carey, J.C., Korf, B., Marks, J., Pyeritz, R.E., Rubenstein, A., Viskochil, D., 1997. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. J. Am. Med. Assoc. 278, 51–57.

Habets, G.G., Scholtes, E.H., Zuydgeest, D., van der Kammen, R.A., Stam, J.C., Berns, A., Collard, J.G., 1994. Identification of an invasion-inducing gene, Tiam-1, that encodes a protein with homology to GDP-GTP exchangers for Rho-like proteins. Cell 77, 537–549.

Hadano, S., Hand, C.K., Osuga, H., Yanagisawa, Y., Otomo, A., Devon, R.S., Miyamoto, N., Showguchi-Miyata, J., Okada, Y., Singaraja, R., Figlewicz, D.A., Kwiatkowski, T., Hosler, B.A., Sagie, T., Skaug, J., Nasir, J., Brown Jr, R.H., Scherer, S.W., Rouleau, G.A., Hayden, M.R., Ikeda, J.E., 2001. A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. Nat. Genet. 29, 166–173.

Hagerman, R.J., 1996. In: Hagerman, R.J., Cronister-Silverman, A. (Eds.). Fragile X Syndrome: Diagnosis, Treatment and Research, John Hopkins Univ. Press, Baltimore, pp. 3–68.

Hall, A., 1998. Rho GTPases and the actin cytoskeleton. Science 279, 509–514.

Hing, H., Xiao, J., Harden, N., Lim, L., Zipursky, S.L., 1999. Pak functions downstream of Dock to regulate photoreceptor axon guidance in Drosophila. Cell 97, 853–863.

Hinton, V.J., Brown, W.T., Wisniewski, K., Rudelli, R.D., 1991. Analysis of neocortex in three males with the fragile X syndrome. Am. J. Med. Genet. 41, 289–294.

Hirano, K., Matsuura, F., Tsukamoto, K., Zhang, Z., Matsuyama, A., Takaishi, K., Komuro, R., Suehiro, T., Yamashita, S., Takai, Y., Matsuzawa, Y., 2000. Decreased expression of a member of the Rho GTPase family, Cdc42Hs, in cells from Tangier disease – the small G protein may play a role in cholesterol efflux. FEBS Lett. 484, 275–279.

Hordijk, P.L., ten Klooster, J.P., van der Kammen, R.A., Michiels, F., Oomen, L.C., Collard, J.G., 1997. Inhibition of invasion of epithelial cells by Tiam1-Rac signaling. Science 278, 1464–1466.

Hu, W., Bellone, C.J., Baldassare, J.J., 1999. RhoA stimulates p27(Kip) degradation through its regulation of cyclin E/CDK2 activity. J. Biol. Chem. 274, 3396–3401.

Hussain, N.K., Jenna, S., Glogauer, M., Quinn, C.C., Wasiak, S., Guipponi, M., Antonarakis, S.E., Kay, B.K., Stossel, T.P., Lamarche-Vane, N., McPherson, P.S., 2001. Endocytic protein intersectin-l regulates actin assembly via Cdc42 and N-WASP. Nat. Cell Biol. 3, 927–932.

Imbert, G., Feng, Y., Nelson, D., Warren, S.T., Mandel, J.-L., 1998. In: Warren, S.T., Wells, R.D. (Eds.). Genetic Instabilities and Hereditary Neurological Diseases, Academic, San Diego, pp. 27–53.

Ishizaki, T., Morishima, Y., Okamoto, M., Furuyashiki, T., Kato, T., Narumiya, S., 2001. Coordination of microtubules and the actin cytoskeleton by the Rho effector mDia1. Nat. Cell Biol. 3, 8–14.

Itoh, K., Yoshioka, K., Akedo, H., Uehata, M., Ishizaki, T., Narumiya, S., 1999. An essential part for Rho-associated kinase in the transcellular invasion of tumor cells. Nat. Med. 5, 221–225.

Jahner, D., Hunter, T., 1991. The ras-related gene rhoB is an immediate-early gene inducible by v-Fps, epidermal growth factor, and platelet-derived growth factor in rat fibroblasts. Mol. Cell. Biol. 11, 3682–3690.

Jalink, K., van Corven, E.J., Hengeveld, T., Morii, N., Narumiya, S., Moolenaar, W.H., 1994. Inhibition of lysophosphatidate- and thrombin-induced neurite retraction and neuronal cell rounding by ADP ribosylation of the small GTP-binding protein Rho. J. Cell Biol. 126, 801–810.

Jin, P., Warren, S.T., 2000. Understanding the molecular basis of fragile X syndrome. Hum. Mol. Genet. 9, 901–908.

Joberty, G., Petersen, C., Gao, L., Macara, I.G., 2000. The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. Nat. Cell Biol. 2, 531–539.

Joneson, T., Bar-Sagi, D., 1999. Suppression of Ras-induced apoptosis by the Rac GTPase. Mol. Cell. Biol. 19, 5892–5901.

Joneson, T., McDonough, M., Bar-Sagi, D., Van Aelst, L., 1996. RAC regulation of actin polymerization and proliferation by a pathway distinct from Jun kinase. Science 274, 1374–1376.

Jordan, P., Brazao, R., Boavida, M.G., Gespach, C., Chastre, E., 1999. Cloning of a novel human Rac1b splice variant with increased expression in colorectal tumors. Oncogene 18, 6835–6839.

Jou, T.S., Schneeberger, E.E., Nelson, W.J., 1998. Structural and functional regulation of tight junctions by RhoA and Rac1 small GTPases. J. Cell Biol. 142, 101–115.

Joyce, D., Bouzahzah, B., Fu, M., Albanese, C., D'Amico, M., Steer, J., Klein, J.U., Lee, R.J., Segall, J.E., Westwick, J.K., Der, C.J., Pestell, R.G., 1999. Integration of Rac-dependent regulation of cyclin D1 transcription through a nuclear factor-kappaB-dependent pathway. J. Biol. Chem. 274, 25245–25249.

Kamai, T., Arai, K., Tsujii, T., Honda, M., Yoshida, K., 2001. Overexpression of RhoA mRNA is associated with advanced stage in testicular germ cell tumour. BJU Int. 87, 227–231.

Kantor, J.D., McCormick, B., Steeg, P.S., Zetter, B.R., 1993. Inhibition of cell motility after nm23 transfection of human and murine tumor cells. Cancer Res. 53, 1971–1973.

Kerkhoff, E., Rapp, U.R., 1998. Cell cycle targets of Ras/Raf signalling. Oncogene 17, 1457–1462.

Khosravi-Far, R., Solski, P.A., Clark, G.J., Kinch, M.S., Der, C.J., 1995. Activation of Rac1, RhoA, and mitogen-activated protein kinases is required for Ras transformation. Mol. Cell. Biol. 15, 6443–6453.

Kim, A.S., Kakalis, L.T., Abdul-Manan, N., Liu, G.A., Rosen, M.K., 2000. Autoinhibition and activation mechanisms of the Wiskott–Aldrich syndrome protein. Nature 404, 151–158.

Kobayashi, K., Kuroda, S., Fukata, M., Nakamura, T., Nagase, T., Nomura, N., Matsuura, Y., Yoshida-Kubomura, N., Iwamatsu, A., Kaibuchi, K., 1998. p140Sra-1 (specifically Rac1-associated protein) is a novel specific target for Rac1 small GTPase. J. Biol. Chem. 273, 291–295.

Kolluri, R., Shehabeldin, A., Peacocke, M., Lamhonwah, A.M., Teichert-Kuliszewska, K., Weissman, S.M., Siminovitch, K.A., 1995. Identification of WASP mutations in patients with Wiskott–Aldrich syndrome and isolated thrombocytopenia reveals allelic heterogeneity at the WAS locus. Hum. Mol. Genet. 4, 1119–1126.

Kourlas, P.J., Strout, M.P., Becknell, B., Veronese, M.L., Croce, C.M., Theil, K.S., Krahe, R., Ruutu, T., Knuutila, S., Bloomfield, C.D., Caligiuri, M.A., 2000. Identification of a gene at 11q23 encoding a guanine nucleotide exchange factor: evidence for its fusion with MLL in acute myeloid leukemia. Proc. Natl. Acad. Sci. USA 97, 2145–2150.

Kroschewski, R., Hall, A., Mellman, I., 1999. Cdc42 controls secretory and endocytic transport to the basolateral plasma membrane of MDCK cells. Nat. Cell Biol. 1, 8–13.

Kutsche, K., Yntema, H., Brandt, A., Jantke, I., Nothwang, H.G., Orth, U., Boavida, M.G., David, D., Chelly, J., Fryns, J.P., Moraine, C., Ropers, H.H., Hamel, B.C., van Bokhoven, H., Gal, A., 2000. Mutations in ARHGEF6, encoding a guanine nucleotide exchange factor for Rho GTPases, in patients with X-linked mental retardation. Nat. Genet. 26, 247–250.

Kwan, S.P., Hagemann, T.L., Radtke, B.E., Blaese, R.M., Rosen, F.S., 1995. Identification of mutations in the Wiskott–Aldrich syndrome gene and characterization of a polymorphic dinucleotide repeat at DXS6940, adjacent to the disease gene. Proc. Natl. Acad. Sci. USA 92, 4706–4710.

Lamarche, N., Tapon, N., Stowers, L., Burbelo, P.D., Aspenstrom, P., Bridges, T., Chant, J., Hall, A., 1996. Rac and Cdc42 induce actin polymerization and G1 cell cycle progression independently of p65PAK and the JNK/SAPK MAP kinase cascade. Cell 87, 519–529.

Lamb, R.F., Roy, C., Diefenbach, T.J., Vinters, H.V., Johnson, M.W., Jay, D.G., Hall, A., 2000. The TSC1 tumour suppressor hamartin regulates

cell adhesion through ERM proteins and the GTPase Rho. Nat. Cell Biol. 2, 281–287.

Leone, A., McBride, O.W., Weston, A., Wang, M.G., Anglard, P., Cropp, C.S., Goepel, J.R., Lidereau, R., Callahan, R., Linehan, W.M., et al., 1991. Somatic allelic deletion of nm23 in human cancer. Cancer Res. 51, 2490–2493.

Lerm, M., Schmidt, G., Aktories, K., 2000. Bacterial protein toxins targeting rho GTPases. FEMS Microbiol Lett. 188, 1–6.

Lin, R., Bagrodia, S., Cerione, R., Manor, D., 1997. A novel Cdc42Hs mutant induces cellular transformation. Curr. Biol. 7, 794–797.

Littlewood Evans, A., Muller, U., 2000. Stereocilia defects in the sensory hair cells of the inner ear in mice deficient in integrin alpha8beta1. Nat. Genet. 24, 424–428.

Liu, A., Cerniglia, G.J., Bernhard, E.J., Prendergast, G.C., 2001a. RhoB is required to mediate apoptosis in neoplastically transformed cells after DNA damage. Proc. Natl. Acad. Sci. USA 98, 6192–6197.

Liu, A.X., Rane, N., Liu, J.P., Prendergast, G.C., 2001b. RhoB is dispensable for mouse development, but it modifies susceptibility to tumor formation as well as cell adhesion and growth factor signaling in transformed cells. Mol. Cell. Biol. 21, 6906–6912.

Luo, L., 2000. Rho GTPases in neuronal morphogenesis. Nat. Rev. Neurosci. 1, 173–180.

Luo, L., Liao, Y.J., Jan, L.Y., Jan, Y.N., 1994. Distinct morphogenetic functions of similar small GTPases: Drosophila Drac1 is involved in axonal outgrowth and myoblast fusion. Genes Dev. 8, 1787–1802.

Lynch, E.D., Lee, M.K., Morrow, J.E., Welsh, P.L., Leon, P.E., King, M.C., 1997. Nonsyndromic deafness DFNA1 associated with mutation of a human homolog of the Drosophila gene diaphanous. Science 278, 1315–1318.

MacDonald, N.J., Freije, J.M., Stracke, M.L., Manrow, R.E., Steeg, P.S., 1996. Site-directed mutagenesis of nm23-H1. Mutation of proline 96 or serine 120 abrogates its motility inhibitory activity upon transfection into human breast carcinoma cells. J. Biol. Chem. 271, 25107–25116.

Manser, E., Leung, T., Salihuddin, H., Zhao, Z.S., Lim, L., 1994. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. Nature 367, 40–46.

Manser, E., Loo, T.H., Koh, C.G., Zhao, Z.S., Chen, X.Q., Tan, L., Tan, I., Leung, T., Lim, L., 1998. PAK kinases are directly coupled to the PIX family of nucleotide exchange factors. Mol. Cell 1, 183–192.

Matus, A., 1999. Postsynaptic actin and neuronal plasticity. Curr. Opin. Neurobiol. 9, 561–565.

Mayo, M.W., Wang, C.Y., Cogswell, P.C., Rogers-Graham, K.S., Lowe, S.W., Der, C.J., Baldwin Jr, A.S., 1997. Requirement of NF-kappaB activation to suppress p53-independent apoptosis induced by oncogenic Ras. Science 278, 1812–1815.

McIntosh, G.G., Anderson, J.J., Milton, I., Steward, M., Parr, A.H., Thomas, M.D., Henry, J.A., Angus, B., Lennard, T.W., Horne, C.H., 1995. Determination of the prognostic value of cyclin D1 overexpression in breast cancer. Oncogene 11, 885–891.

Melzig, J., Rein, K.H., Schafer, U., Pfister, H., Jackle, H., Heisenberg, M., Raabe, T., 1998. A protein related to p21-activated kinase (PAK) that is involved in neurogenesis in the Drosophila adult central nervous system. Curr. Biol. 8, 1223–1226.

Mettouchi, A., Klein, S., Guo, W., Lopez-Lago, M., Lemichez, E., Westwick, J.K., Giancotti, F.G., 2001. Integrin-specific activation of rac controls progression through the G(1) phase of the cell cycle. Mol. Cell 8, 115–127.

Michiels, F., Habets, G.G., Stam, J.C., van der Kammen, R.A., Collard, J.G., 1995. A role for Rac in Tiam1-induced membrane ruffling and invasion. Nature 375, 338–340.

Miki, H., Miura, K., Takenawa, T., 1996. N-WASP, a novel actin-depolymerizing protein, regulates the cortical cytoskeletal rearrangement in a PIP2-dependent manner downstream of tyrosine kinases. EMBO J. 15, 5326–5335.

Miki, H., Sasaki, T., Takai, Y., Takenawa, T., 1998. Induction of filopodium formation by a WASP-related actin- depolymerizing protein N-WASP. Nature 391, 93–96.

Mira, J.P., Benard, V., Groffen, J., Sanders, L.C., Knaus, U.G., 2000. Endogenous, hyperactive Rac3 controls proliferation of breast cancer cells by a p21-activated kinase-dependent pathway. Proc. Natl. Acad. Sci. USA 97, 185–189.

Muller, U., Littlewood-Evans, A., 2001. Mechanisms that regulate mechanosensory hair cell differentiation. Trends Cell Biol. 11, 334–342.

Musch, A., Cohen, D., Kreitzer, G., Rodriguez-Boulan, E., 2001. cdc42 regulates the exit of apical and basolateral proteins from the trans-Golgi network. EMBO J. 20, 2171–2179.

Nagata, K., Driessens, M., Lamarche, N., Gorski, J.L., Hall, A., 1998. Activation of G1 progression, JNK mitogen-activated protein kinase, and actin filament assembly by the exchange factor FGD1. J. Biol. Chem. 273, 15453–15457.

Newsome, T.P., Schmidt, S., Dietzl, G., Keleman, K., Asling, B., Debant, A., Dickson, B.J., 2000. Trio combines with dock to regulate Pak activity during photoreceptor axon pathfinding in Drosophila. Cell 101, 283–294.

O'Bryan, J.P., Mohney, R.P., Oldham, C.E., 2001. Mitogenesis and endocytosis: What's at the INTERSECTIoN? Oncogene 20, 6300–6308.

Olson, M.F., Paterson, H.F., Marshall, C.J., 1998. Signals from Ras and Rho GTPases interact to regulate expression of p21Waf1/Cip1. Nature 394, 295–299.

Orrico, A., Galli, L., Falciani, M., Bracci, M., Cavaliere, M.L., Rinaldi, M.M., Musacchio, A., Sorrentino, V., 2000. A mutation in the pleckstrin homology (PH) domain of the FGD1 gene in an Italian family with faciogenital dysplasia (Aarskog–Scott syndrome). FEBS Lett. 478, 216–220.

Otsuki, Y., Tanaka, M., Yoshii, S., Kawazoe, N., Nakaya, K., Sugimura, H., 2001. Tumor metastasis suppressor nm23H1 regulates Rac1 GTPase by interaction with Tiam1. Proc. Natl. Acad. Sci. USA 98, 4385–4390.

Palazzo, A.F., Cook, T.A., Alberts, A.S., Gundersen, G.G., 2001. mDia mediates Rho-regulated formation and orientation of stable microtubules. Nat. Cell Biol. 3, 723–729.

Pasqualucci, L., Neumeister, P., Goossens, T., Nanjangud, G., Chaganti, R.S., Kuppers, R., Dalla-Favera, R., 2001. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. Nature 412, 341–346.

Pasteris, N.G., Cadle, A., Logie, L.J., Porteous, M.E., Schwartz, C.E., Stevenson, R.E., Glover, T.W., Wilroy, R.S., Gorski, J.L., 1994. Isolation and characterization of the faciogenital dysplasia (Aarskog–Scott syndrome) gene: a putative Rho/Rac guanine nucleotide exchange factor. Cell 79, 669–678.

Peifer, M., Polakis, P., 2000. Wnt signaling in oncogenesis and embryogenesis – a look outside the nucleus. Science 287, 1606–1609.

Perona, R., Montaner, S., Saniger, L., Sanchez-Perez, I., Bravo, R., Lacal, J.C., 1997. Activation of the nuclear factor-kappaB by Rho, CDC42, and Rac-1 proteins. Genes Dev. 11, 463–475.

Polakis, P., 2000. Wnt signaling and cancer. Genes Dev. 14, 1837–1851.

Preudhomme, C., Roumier, C., Hildebrand, M.P., Dallery-Prudhomme, E., Lantoine, D., Lai, J.L., Daudignon, A., Adenis, C., Bauters, F., Fenaux, P., Kerckaert, J.P., Galiegue-Zouitina, S., 2000. Nonrandom 4p13 rearrangements of the RhoH/TTF gene, encoding a GTP- binding protein, in non-Hodgkin's lymphoma and multiple myeloma. Oncogene 19, 2023–2032.

Price, L.S., Collard, J.G., 2001. Regulation of the cytoskeleton by Rho-family GTPases: implications for tumour cell invasion. Semin. Cancer Biol. 11, 167–173.

Pruitt, K., Der, C.J., 2001. Ras and Rho regulation of the cell cycle and oncogenesis. Cancer Lett. 171, 1–10.

Pucharcos, C., Estivill, X., de la Luna, S., 2000. Intersectin 2, a new multimodular protein involved in clathrin-mediated endocytosis. FEBS Lett. 478, 43–51.

Pucharcos, C., Casas, C., Nadal, M., Estivill, X., de la Luna, S., 2001. The human intersectin genes and their spliced variants are differentially expressed. Biochim. Biophys. Acta 1521, 1–11.

Puls, A., Eliopoulos, A.G., Nobes, C.D., Bridges, T., Young, L.S., Hall, A., 1999. Activation of the small GTPase Cdc42 by the inflammatory cyto-

kines TNF(alpha) and IL-1, and by the Epstein–Barr virus transforming protein LMP1. J. Cell Sci. 112, 2983–2992.

Qiu, R.G., Chen, J., Kirn, D., McCormick, F., Symons, M., 1995a. An essential role for Rac in Ras transformation. Nature 374, 457–459.

Qiu, R.G., Chen, J., McCormick, F., Symons, M., 1995b. A role for Rho in Ras transformation. Proc. Natl. Acad. Sci. USA 92, 11781–11785.

Qiu, R.G., Abo, A., McCormick, F., Symons, M., 1997. Cdc42 regulates anchorage-independent growth and is necessary for Ras transformation. Mol. Cell. Biol. 17, 3449–3458.

Qiu, R.G., Abo, A., Steven Martin, G., 2000. A human homolog of the *C. elegans* polarity determinant Par-6 links Rac and Cdc42 to PKCzeta signaling and cell transformation. Curr. Biol. 10, 697–707.

Rameh, L.E., Arvidsson, A., Carraway 3rd, K.L., Couvillon, A.D., Rathbun, G., Crompton, A., VanRenterghem, B., Czech, M.P., Ravichandran, K.S., Burakoff, S.J., Wang, D.S., Chen, C.S., Cantley, L.C., 1997. A comparative analysis of the phosphoinositide binding specificity of pleckstrin homology domains. J. Biol. Chem. 272, 22059–22066.

Reiss, A.L., Aylward, E., Freund, L.S., Joshi, P.K., Bryan, R.N., 1991. Neuroanatomy of fragile X syndrome: the posterior fossa. Ann. Neurol. 29, 26–32.

Reiss, A.L., Lee, J., Freund, L., 1994. Neuroanatomy of fragile X syndrome: the temporal lobe. Neurology 44, 1317–1324.

Reuther, G.W., Lambert, Q.T., Booden, M.A., Wennerberg, K., Becknell, B., Marcucci, G., Sondek, J., Caligiuri, M.A., Der, C.J., 2001. Leukemia-associated Rho guanine nucleotide exchange factor, a Dbl family protein found mutated in leukemia, causes transformation by activation of RhoA. J. Biol. Chem. 276, 27145–27151.

Richardson, G.P., Forge, A., Kros, C.J., Marcotti, W., Becker, D., Williams, D.S., Thorpe, J., Fleming, J., Brown, S.D., Steel, K.P., 1999. A missense mutation in myosin VIIA prevents aminoglycoside accumulation in early postnatal cochlear hair cells. Ann. N. Y. Acad. Sci. 884, 110–124.

Ridley, A.J., 2001. Rho proteins: linking signaling with membrane trafficking. Traffic 2, 303–310.

Rosen, D.R., 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 364, 362.

Rudelli, R.D., Brown, W.T., Wisniewski, K., Jenkins, E.C., Laure-Kamionowska, M., Connell, F., Wisniewski, H.M., 1985. Adult fragile X syndrome. Clinico-neuropathologic findings. Acta Neuropathol. 67, 289–295.

Ruggieri, R., Chuang, Y.Y., Symons, M., 2001. The small GTPase Rac suppresses apoptosis caused by serum deprivation in fibroblasts. Mol. Med. 7, 293–300.

Sahai, E., Ishizaki, T., Narumiya, S., Treisman, R., 1999. Transformation mediated by RhoA requires activity of ROCK kinases. Curr. Biol. 9, 136–145.

Salim, K., Bottomley, M.J., Querfurth, E., Zvelebil, M.J., Gout, I., Scaife, R., Margolis, R.L., Gigg, R., Smith, C.I., Driscoll, P.C., Waterfield, M.D., Panayotou, G., 1996. Distinct specificity in the recognition of phosphoinositides by the pleckstrin homology domains of dynamin and Bruton's tyrosine kinase. EMBO J. 15, 6241–6250.

Sander, E.E., van Delft, S., ten Klooster, J.P., Reid, T., van der Kammen, R.A., Michiels, F., Collard, J.G., 1998. Matrix-dependent Tiam1/Rac signaling in epithelial cells promotes either cell-cell adhesion or cell migration and is regulated by phosphatidylinositol 3-kinase. J. Cell Biol. 143, 1385–1398.

Sanders, L.C., Matsumura, F., Bokoch, G.M., de Lanerolle, P., 1999. Inhibition of myosin light chain kinase by p21-activated kinase. Science 283, 2083–2085.

Schenck, A., Bardoni, B., Moro, A., Bagni, C., Mandel, J.L., 2001. A highly conserved protein family interacting with the fragile X mental retardation protein (FMRP) and displaying selective interactions with FMRP-related proteins FXR1P and FXR2P. Proc. Natl. Acad. Sci. USA 98, 8844–8849.

Schmitz, A.A., Govek, E.E., Bottner, B., Van Aelst, L., 2000. Rho GTPases: signaling, migration, and invasion. Exp. Cell Res. 261, 1–12.

Schnelzer, A., Prechtel, D., Knaus, U., Dehne, K., Gerhard, M., Graeff, H., Harbeck, N., Schmitt, M., Lengyel, E., 2000. Rac1 in human breast cancer: overexpression, mutation analysis, and characterization of a new isoform, Rac1b. Oncogene 19, 3013–3020.

Schwartz, C.E., Gillessen-Kaesbach, G., May, M., Cappa, M., Gorski, J., Steindl, K., Neri, G., 2000. Two novel mutations confirm FGD1 is responsible for the Aarskog syndrome. Eur. J. Hum. Genet. 8, 869–874.

Sells, M.A., Boyd, J.T., Chernoff, J., 1999. p21-activated kinase 1 (Pak1) regulates cell motility in mammalian fibroblasts. J. Cell Biol. 145, 837–849.

Shaw, R.J., Paez, J.G., Curto, M., Yaktine, A., Pruitt, W.M., Saotome, I., O'Bryan, J.P., Gupta, V., Ratner, N., Der, C.J., Jacks, T., McClatchey, A.I., 2001. The Nf2 tumor suppressor, merlin, functions in Rac-dependent signaling. Dev. Cell 1, 63–72.

Sherman, L., Xu, H.M., Geist, R.T., Saporito-Irwin, S., Howells, N., Ponta, H., Herrlich, P., Gutmann, D.H., 1997. Interdomain binding mediates tumor growth suppression by the NF2 gene product. Oncogene 15, 2505–2509.

Sherr, C.J., Roberts, J.M., 1999. CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev. 13, 1501–1512.

Singh, R., Wang, B., Shirvaikar, A., Khan, S., Kamat, S., Schelling, J.R., Konieczkowski, M., Sedor, J.R., 1999. The IL-1 receptor and Rho directly associate to drive cell activation in inflammation. J. Clin. Invest. 103, 1561–1570.

Siomi, H., Siomi, M.C., Nussbaum, R.L., Dreyfuss, G., 1993. The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. Cell 74, 291–298.

Sittler, A., Devys, D., Weber, C., Mandel, J.L., 1996. Alternative splicing of exon 14 determines nuclear or cytoplasmic localisation of fmr1 protein isoforms. Hum. Mol. Genet. 5, 95–102.

Somlyo, A.V., Bradshaw, D., Ramos, S., Murphy, C., Myers, C.E., Somlyo, A.P., 2000. Rho-kinase inhibitor retards migration and in vivo dissemination of human prostate cancer cells. Biochem. Biophys. Res. Commun. 269, 652–659.

Srivastava, S.K., Wheelock, R.H., Aaronson, S.A., Eva, A., 1986. Identification of the protein encoded by the human diffuse B-cell lymphoma (dbl) oncogene. Proc. Natl. Acad. Sci. USA 83, 8868–8872.

Stebbins, C.E., Galan, J.E., 2001. Structural mimicry in bacterial virulence. Nature 412, 701–705.

Stipp, C.S., Hemler, M.E., 2000. Transmembrane-4-superfamily proteins CD151 and CD81 associate with alpha 3 beta 1 integrin, and selectively contribute to alpha 3 beta 1-dependent neurite outgrowth. J. Cell Sci. 113, 1871–1882.

Sugihara, K., Nakatsuji, N., Nakamura, K., Nakao, K., Hashimoto, R., Otani, H., Sakagami, H., Kondo, H., Nozawa, S., Aiba, A., Katsuki, M., 1998. Rac1 is required for the formation of three germ layers during gastrulation. Oncogene 17, 3427–3433.

Sulciner, D.J., Irani, K., Yu, Z.X., Ferrans, V.J., Goldschmidt-Clermont, P., Finkel, T., 1996. rac1 regulates a cytokine-stimulated, redox-dependent pathway necessary for NF-kappaB activation. Mol. Cell. Biol. 16, 7115–7121.

Suwa, H., Ohshio, G., Imamura, T., Watanabe, G., Arii, S., Imamura, M., Narumiya, S., Hiai, H., Fukumoto, M., 1998. Overexpression of the rhoC gene correlates with progression of ductal adenocarcinoma of the pancreas. Br. J. Cancer 77, 147–152.

Suzuki, A., Yamanaka, T., Hirose, T., Manabe, N., Mizuno, K., Shimizu, M., Akimoto, K., Izumi, Y., Ohnishi, T., Ohno, S., 2001. Atypical protein kinase C is involved in the evolutionarily conserved par protein complex and plays a critical role in establishing epithelia-specific junctional structures. J. Cell Biol. 152, 1183–1196.

Symons, M., 1996. Rho family GTPases: the cytoskeleton and beyond. Trends Biochem. Sci. 21, 178–181.

Symons, M., Settleman, J., 2000. Rho family GTPases: more than simple switches. Trends Cell Biol. 10, 415–419.

Symons, M., Derry, J.M., Karlak, B., Jiang, S., Lemahieu, V., McCormick, F., Francke, U., Abo, A., 1996. Wiskott–Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization. Cell 84, 723–734.

Takahashi, K., Sasaki, T., Mammoto, A., Takaishi, K., Kameyama, T., Tsukita, S., Takai, Y., 1997. Direct interaction of the Rho GDP dissociation inhibitor with ezrin/radixin/moesin initiates the activation of the Rho small G protein. J. Biol. Chem. 272, 23371–23375.

Takahashi, K., Sasaki, T., Mammoto, A., Hotta, I., Takaishi, K., Imamura, H., Nakano, K., Kodama, A., Takai, Y., 1998. Interaction of radixin with Rho small G protein GDP/GTP exchange protein Dbl. Oncogene 16, 3279–3284.

Takeshima, H., Komazaki, S., Nishi, M., Iino, M., Kangawa, K., 2000. Junctophilins: a novel family of junctional membrane complex proteins. Mol. Cell 6, 11–22.

Tamanini, F., Bontekoe, C., Bakker, C.E., van Unen, L., Anar, B., Willemsen, R., Yoshida, M., Galjaard, H., Oostra, B.A., Hoogeveen, A.T., 1999. Different targets for the fragile X-related proteins revealed by their distinct nuclear localizations. Hum. Mol. Genet. 8, 863–869.

Tang, Y., Chen, Z., Ambrose, D., Liu, J., Gibbs, J.B., Chernoff, J., Field, J., 1997. Kinase-deficient Pak1 mutants inhibit Ras transformation of Rat-1 fibroblasts. Mol. Cell. Biol. 17, 4454–4464.

Tao, W., Pennica, D., Xu, L., Kalejta, R.F., Levine, A.J., 2001. Wrch-1, a novel member of the Rho gene family that is regulated by Wnt- 1. Genes Dev. 15, 1796–1807.

Taya, S., Inagaki, N., Sengiku, H., Makino, H., Iwamatsu, A., Urakawa, I., Nagao, K., Kataoka, S., Kaibuchi, K., 2001. Direct interaction of insulin-like growth factor-1 receptor with leukemia-associated RhoGEF. J. Cell Biol. 155, 809–820.

Tiwari-Woodruff, S.K., Buznikov, A.G., Vu, T.Q., Micevych, P.E., Chen, K., Kornblum, H.I., Bronstein, J.M., 2001. OSP/claudin-11 forms a complex with a novel member of the tetraspanin super family and beta1 integrin and regulates proliferation and migration of oligodendrocytes. J. Cell Biol. 153, 295–305.

Tran Quang, C., Gautreau, A., Arpin, M., Treisman, R., 2000. Ezrin function is required for ROCK-mediated fibroblast transformation by the Net and Dbl oncogenes. EMBO J. 19, 4565–4576.

Utech, M., Hobbel, G., Rust, S., Reinecke, H., Assmann, G., Walter, M., 2001. Accumulation of RhoA, RhoB, RhoG, and Rac1 in fibroblasts from Tangier disease subjects suggests a regulatory role of Rho family proteins in cholesterol efflux. Biochem. Biophys. Res. Commun. 280, 229–236.

Van Aelst, L., D'Souza-Schorey, C., 1997. Rho GTPases and signaling networks. Genes Dev 11, 2295–2322.

van Golen, K.L., Wu, Z.F., Qiao, X.T., Bao, L.W., Merajver, S.D., 2000. RhoC GTPase, a novel transforming oncogene for human mammary epithelial cells that partially recapitulates the inflammatory breast cancer phenotype. Cancer Res. 60, 5832–5838.

Villa, A., Notarangelo, L., Macchi, P., Mantuano, E., Cavagni, G., Brugnoni, D., Strina, D., Patrosso, M.C., Ramenghi, U., Sacco, M.G., et al., 1995. X-linked thrombocytopenia and Wiskott–Aldrich syndrome are allelic diseases with mutations in the WASP gene. Nat. Genet. 9, 414–417.

Watanabe, N., Madaule, P., Reid, T., Ishizaki, T., Watanabe, G., Kakizuka, A., Saito, Y., Nakao, K., Jockusch, B.M., Narumiya, S., 1997. p140mDia, a mammalian homolog of Drosophila diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. EMBO J. 16, 3044–3056.

Watanabe, N., Kato, T., Fujita, A., Ishizaki, T., Narumiya, S., 1999. Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. Nat. Cell Biol. 1, 136–143.

Weber, J.D., Hu, W., Jefcoat Jr, S.C., Raben, D.M., Baldassare, J.J., 1997. Ras-stimulated extracellular signal-related kinase 1 and RhoA activities coordinate platelet-derived growth factor-induced G1 progression through the independent regulation of cyclin D1 and p27. J. Biol. Chem. 272, 32966–32971.

Weiler, I.J., Irwin, S.A., Klintsova, A.Y., Spencer, C.M., Brazelton, A.D., Miyashiro, K., Comery, T.A., Patel, B., Eberwine, J., Greenough, W.T., 1997. Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. Proc. Natl. Acad. Sci. USA 94, 5395–5400.

Welch, M.D., 1999. The world according to Arp: regulation of actin nucleation by the Arp2/3 complex. Trends Cell Biol. 9, 423–427.

Welsh, C.F., Roovers, K., Villanueva, J., Liu, Y., Schwartz, M.A., Assoian, R.K., 2001. Timing of cyclin D1 expression within G1 phase is controlled by Rho. Nat. Cell Biol. 3, 950–957.

Wengler, G.S., Notarangelo, L.D., Berardelli, S., Pollonni, G., Mella, P., Fasth, A., Ugazio, A.G., Parolini, O., 1995. High prevalence of nonsense, frame shift, and splice-site mutations in 16 patients with full-blown Wiskott–Aldrich syndrome. Blood 86, 3648–3654.

Westwick, J.K., Lambert, Q.T., Clark, G.J., Symons, M., Van Aelst, L., Pestell, R.G., Der, C.J., 1997. Rac regulation of transformation, gene expression, and actin organization by multiple, PAK-independent pathways. Mol. Cell. Biol. 17, 1324–1335.

Whitehead, I.P., Lambert, Q.T., Glaven, J.A., Abe, K., Rossman, K.L., Mahon, G.M., Trzaskos, J.M., Kay, R., Campbell, S.L., Der, C.J., 1999. Dependence of Dbl and Dbs transformation on MEK and NF-kappaB activation. Mol. Cell. Biol. 19, 7759–7770.

Wu, W.J., Erickson, J.W., Lin, R., Cerione, R.A., 2000. The gamma-subunit of the coatomer complex binds Cdc42 to mediate transformation. Nature 405, 800–804.

Yang, N., Higuchi, O., Ohashi, K., Nagata, K., Wada, A., Kangawa, K., Nishida, E., Mizuno, K., 1998. Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. Nature 393, 809–812.

Yang, F.C., Kapur, R., King, A.J., Tao, W., Kim, C., Borneo, J., Breese, R., Marshall, M., Dinauer, M.C., Williams, D.A., 2000. Rac2 stimulates Akt activation affecting BAD/Bcl-XL expression while mediating survival and actin function in primary mast cells. Immunity 12, 557–568.

Yang, Y., Hentati, A., Deng, H.X., Dabbagh, O., Sasaki, T., Hirano, M., Hung, W.Y., Ouahchi, K., Yan, J., Azim, A.C., Cole, N., Gascon, G., Yagmour, A., Ben-Hamida, M., Pericak-Vance, M., Hentati, F., Siddique, T., 2001. The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. Nat. Genet. 29, 160–165.

Yu, Q., Geng, Y., Sicinski, P., 2001. Specific protection against breast cancers by cyclin D1 ablation. Nature 411, 1017–1021.

Zemni, R., Bienvenu, T., Vinet, M.C., Sefiani, A., Carrie, A., Billuart, P., McDonell, N., Couvert, P., Francis, F., Chafey, P., Fauchereau, F., Friocourt, G., Portes, V., Cardona, A., Frints, S., Meindl, A., Brandau, O., Ronce, N., Moraine, C., Bokhoven, H., Ropers, H.H., Sudbrak, R., Kahn, A., Fryns, J.P., Beldjord, C., et al., 2000. A new gene involved in X-linked mental retardation identified by analysis of an X;2 balanced translocation. Nat. Genet. 24, 167–170.

Zheng, Y., Fischer, D.J., Santos, M.F., Tigyi, G., Pasteris, N.G., Gorski, J.L., Xu, Y., 1996. The faciogenital dysplasia gene product FGD1 functions as a Cdc42Hs- specific guanine-nucleotide exchange factor. J. Biol. Chem. 271, 33169–33172.

Zhu, Q., Zhang, M., Blaese, R.M., Derry, J.M., Junker, A., Francke, U., Chen, S.H., Ochs, H.D., 1995. The Wiskott–Aldrich syndrome and X-linked congenital thrombocytopenia are caused by mutations of the same gene. Blood 86, 3797–3804.